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SPECIAL LISTING

CURRENT CANCER RESEARCH

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CLINICAL AND EXPERIMENTAL CANCER IMMUNOTHERAPY

APRIL 1981

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INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB)

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Dear Cancer Researcher:

This **SPECIAL LISTING** of Current Cancer Research Projects is a publication of the INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB) Program of the National Cancer Institute. Each **LISTING** contains descriptions of ongoing projects in one selected cancer research area. Approximately 50 different cancer research areas are covered by a separate **SPECIAL LISTING**.

The present series of **SPECIAL LISTINGS** is a third edition, and begins with number 201. This series will update the second edition which included **SPECIAL LISTINGS** numbered 101 through 147. Depending on the total number and types of cancer research summaries received, the third edition will have at least 47 titles, and possibly a few more. In most cases, each third edition **SPECIAL LISTING** will replace the exact subject area of a second edition **LISTING** (SL 201 replacing SL 101, etc.). Whenever the number and variety of summaries requires a change in this procedure, a notation will be made in the specific **LISTINGS** affected.

The purpose of these **LISTINGS** is to promote and facilitate the exchange of information among cancer scientists. The project descriptions in the **LISTINGS** are provided by cancer scientists from more than 50 different countries. Researchers are urged to prepare accurate summaries which will be rapidly processed for inclusion in the CANCERPROJ data base as well as future **SPECIAL LISTINGS**.

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IF YOU ARE A PRINCIPAL INVESTIGATOR and find your summary in this **SPECIAL LISTING** to be out of date, inaccurate, or incomplete, please prepare and send a new summary to the CCRESPAC PROJECT OFFICER at the address below as soon as possible. Please also check the address of your project for completeness and accuracy. If the addresses of co-investigators are different from the Principal Investigator's, add their addresses at the bottom of the new project summary. A copy of the Project Registration Form can be found in the back of this **LISTING**.

ICRDB Program Office
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Descriptions of more than 20,000 other current cancer-related projects can be retrieved via online computer terminals at more than 800 locations in the U.S. and 12 countries. Any organization linked to the MEDLARS/MEDLINE System at the National Library of Medicine has access to this online file of research project summaries called "CANCERPROJ."

RESEARCH AREAS

	Abstract Number
I. IMMUNOTHERAPY OF HUMAN CANCER	1
A. IMMUNOTHERAPY OF MELANOMA	1
B. IMMUNOTHERAPY OF LEUKEMIAS, LYMPHOMAS, SARCOMAS, AND MYELOMAS	18
C. IMMUNOTHERAPY OF LUNG CANCER	47
D. IMMUNOTHERAPY OF BREAST CANCER	68
E. IMMUNOTHERAPY OF GENITOURINARY CANCERS	81
F. IMMUNOTHERAPY OF GASTROINTESTINAL CANCERS	97
G. IMMUNOTHERAPY OF HEAD AND NECK CANCERS	112
H. IMMUNOTHERAPY OF OTHER AND MULTIPLE CANCERS	120
I. OTHER CLINICAL IMMUNOTHERAPY STUDIES AND BROAD PROGRAMS	142
II. PRECLINICAL ASPECTS OF CANCER IMMUNOTHERAPY	166
A. IMMUNOPOTENTIATORS	166
1. BCG	166
2. C. PARVUM	188
3. OTHER MICROBIAL ADJUVANTS	199
4. OTHER ADJUVANTS	213
B. ACTIVE IMMUNOTHERAPY WITH TUMOR CELLS, TUMOR CELL COMPONENTS, OR OTHER CELLS	230
C. ADOPTIVE IMMUNIZATION	244
1. MONOCLONAL AND OTHER ANTIBODIES	244
2. IMMUNOCOMPETENT CELLS	257
3. TRANSFER FACTOR AND IMMUNE RNA	276
D. EXPERIMENTAL IMMUNOTHERAPY OF SPECIFIC CANCERS	290
1. EXPERIMENTAL IMMUNOTHERAPY OF LEUKEMIAS, LYMPHOMAS, AND SARCOMAS	290
2. EXPERIMENTAL IMMUNOTHERAPY OF OTHER SPECIFIC CANCERS	299
E. OTHER EXPERIMENTAL IMMUNOTHERAPY STUDIES AND BROAD PROGRAMS	312

Investigator Index

NOTE: If you are a Principal Investigator with a research project registered with the ICRDB, you are entitled to two complimentary subscriptions of CANCERGRAMS. If you are NOT receiving CANCERGRAMS, be sure to mention this in your next correspondence with the Program.

CURRENT CANCER RESEARCH

I. IMMUNOTHERAPY OF HUMAN CANCER

A. IMMUNOTHERAPY OF MELANOMA

1. ASSAY OF IMMUNE RESPONSE TO MELANOMA-ASSOCIATED ANTIGENS IN PATIENTS RECEIVING IMMUNOTHERAPY WITH MELANOMA TUMOR CELL VACCINE

Dent, P. B., Singal, D. P., Liao, S. K., McMaster University, Dept. of Pediatrics, *1200 Main St. W., Hamilton, Ontario,* L8S 4L8, Canada

OBJECTIVES: There are now several reports that immunization of patients with tumor vaccines results in the development of antibodies against antigens which are shared by tumors of the same histological type as that used for immunization. The nature of the antigens involved in this antibody response and also the relationship of the antibody response to clinical outcome have not been well documented. We have developed a sensitive serological assay for antibodies to cultured human tumor cells which requires very small volumes of reagents, thus permitting extensive specificity tests to be performed. Furthermore, we have access to sera from a large immunotherapy study of patients with malignant melanoma conducted at the National Cancer Institute in the United States, which will permit us to explore the following questions: 1. Do patients immunized with allogeneic melanoma cells produce antibodies against antigens found on the surface of these cells? 2. Can we distinguish the specificity of this response between histocompatibility antigens, histiotypic antigens such as those found on normal melanocytes, antigens shared between melanoma cells and other cells derived from the same embryological source, fetal tissues, antigens shared by melanoma cells and antigens with a more restricted distribution within the same histological classification, i.e. melanoma? 3. Is there a characteristc pattern of response which is associated with the clinical outcome of the patient?

APPROACH: Sera will be obtained from 200 patients with high risk primary malignant melanoma rendered disease-free and randomized to receive no further therapy, adjuvant chemotherapy, BCG immunotherapy or immunotherapy with BCG and a melanoma cell vaccine consisting of three allogeneic melanoma cell lines. The sera will be tested for reactivity against 10 different melanoma cell lines by a sensitive miniaturized mixed hemadsorption assay. Specificity of reactivity will be determined by absorption with pooled human platelets, non-melanoma cell lines, fetal calf serum, fetal tisue homogenates as well as direct testing against normal melanocytes and other neuroectodermally derived cell lines. Reactivity will be correlated with clinical outcome.

2. EXPERIMENTAL THERAPY OF HUMAN MELANOMA WITH VACCINES

Oettgen, H. F., Old, L. J., Livingston, P. O., Albino, A. P., Houghton, A. N., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

While the knowledge of microbial antigens has permitted the successful development of vaccines against infections, lack of comparable knowledge of human cancer antigens has hampered the development of human cancer vaccines of defined cancer-restricted antigenicity. Recent advances based on autologous serological typing of cultured melanoma cells have led to the identification of two classes of melanoma-restricted cell surface antigens, Class I, detected only on the autologous melanoma cell, and Class II, shared by other melanomas. We propose to test, in a sequential fashion, various types of melanoma vaccines expressing Class I/II antigens to determine conditions for maximal immunogenicity. In initial tests of vaccines prepared from irradiated autologous cultured melanoma cells, antibody to Class I/II antigens was rarely induced or augmented. Therefore, we intend to test vaccines carrying additional antigenic determinants, with the expectation that these determinants will provide 'help' for the recognition of Class I/II melanoma antigens. The vaccines will be prepared from (1) irradiated cultured allogeneic melanoma cells expressing cross-reacting melanoma cell surface antigens, (2) irradiated cultured autologous melanoma cells infected with vesicular stomatitis virus, (3) irradiated chemically modified autologous melanoma cells, (4) irradiated intraspecies or interspecies hybrids expressing Class I/II antigens, and (5) immunogenic forms of isolated Class I/II antigens. The relevant immunogenicity of the vaccines will be determined by comprehensive analysis of the patients' antibody response to Class I/II antigens. This is an experimental pilot study concerned with analysis of the serological response to vaccine administration. If a change in serological reactivity to Class I/II melanoma antigens is induced with some regularity, a clinical immunotherapy trial will be designed.

3. COORDINATING CENTER FOR THE CLINICAL STUDY OF MELANOMA

Veronesi, U., Istituto Nazionale per lo Studio e la Cura dei Tumori, *1 Via G Venezian, 20133 Milan, Italy*

Adjuvant study in operable melanoma: Patients with involvement of regional nodes and patients with primary melanoma of the trunk, even in the absence of regional positive nodes, are included. Adjuvant treatment consists of DTIC, BCG and DTIC-BCG. 180 patients entered into the study. It is still too early to provide evidence for a difference in the relapse rate between the treatment groups. Chemotherapy and chemotherapy plus specific immunotherapy in advanced melanoma: All patients with distant metastases (except brain metastases) are included.

4. IMMUNOREACTIVITY OF SURGICALLY TREATED MELANOMA PATIENTS IN THE COURSE OF BCG VACCINATION

Machalek, J., Lochman, I., Kysela, T., Beska, F., Krajaska Hygienicka Stanice, Dept. of Immunology, *Partyzanske Nam. 7, Ostrava 72892, Czechoslovakia*

Over a long period of time BCG vaccine was repeatedly given to surgically treated melanoma patients and their reactivity in the resting disease phase was tested. The vaccination program, the selection of patients for the BCG vaccination and the applicability of tests to estimate BCG therapy effects were studied.

APPROACH: Leucocytes and differential counting, T-lymphocyte counting by E-rosette test, lymphocyte functions according to their response to PHA in lymphocyte transformation test, phagocytic activity of polymorphonuclears by NBT and INT tests, complement level tests, immunoglobulins A,G,M and D, C-reactive protein-level tests and latex-fixation test for detecting rheumatoid-factor type antiglobulins were employed.

PROGRESS: During prolonged BCG application some deviations of immunity parameters were observed. Their causes are not yet clear, but the influence of BCG vaccination on them cannot be excluded from considerations. Immunological response comparison of living (25) and deceased (13) patients showed no remarkable differences except for a higher incidence of rheumatoid-factor type antiglobulins detected by the latex-fixation test in the group of deceased patients. These examinations were carried out using lymphocyte counting in the M-

rosette test, the Pegikem test for immune complexes level, the test of lymphocyte response to PPD, the Middlebrook-Dubos test of antibody level to PPD and the LAI-test of melanoma antigen response, and the examinations are continuing.

5. STUDY OF DEPRESSED IMMUNOCAPACITY OF MALIGNANT MELANOMA PATIENTS AND RESULTS OF IMMUNOTHERAPY

The, T. H., van der Giessen, M., Koops, H. S., Rijksuniversiteit te Groningen, City & University Hospital, Dept. of Medicine, *Oostersingel 59, Groningen, Netherlands*

OBJECTIVE: Analysis of humoral and cellular factors associated with depressed lymphocyte functions. Influence of immunotherapy on these parameters and patients' prognosis.

APPROACH AND PROGRESS: Primary test immunogens alpha-haemocyanin from the Helix Pomatia and dinitrochlorobenzene are used for testing cellular and humoral immunocapacity by skin tests and in vitro lymphocyte blastogenesis responses (E. Bleumink et al., Cancer 33, 911, 1974 and G.C. de Gast et al., Acta Med. Scand. 197, 303, 1973). Immunoglobulin class-specific anti-haemocyanin antibody responses are measured by an ELISA method (J. Weits et al., Clin. Exp. Immunol. 32, 443, 1978). Results showed a relationship between immune-reactivity, tumor staging and prognosis after tumor surgery (De Gast et al., Cancer 36, 1289, 1975 and Clin. Exp. Immunol. 23, 491, 1976). The role of monocytes is currently being investigated. This is also the case for immunotherapy with BCG in combination with intensive plasmapheresis and its effects on immunosuppressive serum factors, immune complexes and lymphocyte functions. The clinical response to treatment will also be evaluated in relation to these immunological aspects.

6. LABORATORY MONITORING OF IMMUNOTHERAPY OF MALIGNANT MELANOMA (MALE TRUNCAL)

Hobbs, J. R., Hiles, R., Reid, C., Byrom, N., University of London, Westminster Medical School, Dept. of Chemical Pathology/Surgery, *17 Horseferry Rd., London SW1P 2AR, England, United Kingdom*

OBJECTIVE: A trial of immunostimulant therapy in a subgroup of patients with an appalling prognosis, which should allow assessment within 3 years. Apart from clinical monitoring, the laboratory will assess whether immunological parameters improve in the immunostimulated group as compared to placebo controls.

APPROACH: Males newly presenting with malanomas of the trunk will be carefully graded clinically and histopathologically (Clark's) and some time during the two weeks following surgery will have the following parameters assessed before beginning therapy: malanoma specific protein excretion, T-cells (E-rosetting in medium only and following the addition of immunostimulants), mixed lymphocyte reaction and response to candida immunogen, total lymphocyte count, total neutrophil count, etc. The patients will be randomly allocated to either immunostimulant or placebo.

PROGRESS: The initial chosen doses of immunostimulant were effective by laboratory parameters and indeed produced objective regression of tumor in 3 out of 18 patients. However, in all cases the fully adequate dose of Azimexon caused severe anemia of a hemolytic type not yet identified (Coombs' negative). The laboratory studies revealed that the best immunostimulation and marrow stimulation occurred at doses around 25 micrograms/ml, which requires a single IV injection of 400 mg and may be achieved by a single oral dose of 600 mg. At these doses interfeon is released from the patient's leukocytes. At present it appears that when doses are administered only once a week, the anemia is transient and can be tolerated. The trial has, therefore, commenced with oral dosage at 600 mg once a week.

BIBLIOGRAPHIC REFERENCES: Byrom, N., Retsas, S., Dean, A.J., and Hobbs, J.R. (1978), The importance of dose of thymosin for the in vitro induction of T-lymphocytes from patients with solid tumors. Clin. Oncol. 4, 25-34. Byrom, N., et al. (1978), Thymosin-inducible lymphocytes in the peripheral blood of patients with malignant melanoma. Cancer Treatment Reports 62, 1769-1774.

7. IMMUNOTHERAPY OF HUMAN CANCER

Terry, W. D., Hodes, R. J., Rosenberg, S. A., Fisher, R. I., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

A controlled, randomized trial comparing immunotherapy with chemotherapy in stage I and stage II malignant melanoma has been initiated. A total of 169 patients have entered the trial, but it is too early to evaluate the results.

BIBLIOGRAPHIC REFERENCES: Terry, W.D.: BCG in the treatment and prevention of human cancer. In Sela, M. (Ed.): Proceedings of the Study Week on the Role of Non-Specific Immunity in the Prevention and Treatment of Cancer, vatican City, Pontificia Academia Scientiarvm, 1979, pp. 533-577. Terry, W.D. and Yamamura, Y. (Eds.): Developments in Immunology, Vol. 6 Immunobiology and Immunotherapy of Cancer. New York, NY, Elsevier North-Holland, Inc., 1979, 424 pp.

8. MALIGNANT MELANOMA - A CONTROLLED STUDY WITH CONTINUED CHEMOTHERAPY, TOGETHER WITH IMMUNOTHERAPY AS AN AID TO SURGERY

Priario, J. C., D'Auria, A. E., Mate, M., de los Santos, J., Hospital de Clinicas Manuel Quintela, Clinical Quirurgica B, *Avenida Italia s/n, y Las Heras Planta baja, Montevideo, Uruguay*

This project is being carried out as a project of the International Group for the Clinical Study of Melanoma, an agency of WHO.

OBJECTIVE: To determine if prolonged chemotherapy, or prolonged immunotherapy with chemotherapy, shows better results in patients treated exclusively with surgery.

APPROACH: DTIC is the agent in chemotherapy, and BCG is used in the immunotherapy. The patients were initially divided into previously treated and untreated cases. There is a second division according to sex. Finally, a random selection is made, with the following groups: 1) Surgery only, 2) surgery plus chemotherapy, 3) surgery plus immunotherapy, 4) surgery plus chemotherapy and immunotherapy. The DTIC dose is 200 mg/m2. Immunotherapy with BCG is carried out through scarification; the BCG originates from the Pasteur Institute.

PROGRESS: An increase in the disease-free period in patients treated with adjuvants in comparison with the control groups has been shown after 30 months. No increase in the survival rates is reflected when comparing the different arms of the study.

9. AN ADJUVANT STUDY OF COMBINATION CHEMOTHERAPY WITH OR WITHOUT IMMUNOTHERAPY IN HIGH RISK MELANOMA PATIENTS

Bottomley, R. H., Grozea, P. N., Hussein, K. K., Murray, J. L., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Hematology/Oncology Section, *921 N.E. 13th St., Oklahoma City, Oklahoma,* 73104, United States of America

The purpose of this study is to determine the efficacy of C. parvum immunotherapy in preventing recurrence of disease and prolonging overall survival in malignant melanoma patients who have undergone complete resection of the primary lesion without evidence of residual disease, and in whom the likelihood of recurrence is high, based on clinical and/or pathological characteristics of the tumor. Another purpose is to determine the efficacy of chemotherapy with high dose DTIC alone versus chemotherapy with C. parvum plus DTIC in prolonging the disease-free interval and survival in patients with regional or metastatic disease which is amenable to surgical excision.

To be eligible for this study patients must have histologically confirmed diagnosis of malignant melanoma and not have been previously treated with chemotherapy or radiotherapy. Also, patients must have adequate renal and hepatic function. Patients must begin therapy within 6 weeks of surgical excision of active disease.

Patients will be classified as Class I (localized disease) or Class II (regional or resected disease or disease with poor prognostic criteria). Patients with Class I disease will be randomized between C. parvum and no treatment. Class II patients will be randomized between high dose DTIC alone and high dose DTIC plus C. parvum.

10. CHEMO-IMMUNOTHERAPY OF MALIGNANT MELANOMA

Hande, K. R., Oldham, R. K., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *1310 24th Ave. S., Nashville, Tennessee,* 37203, United States of America

The objective of this project is to determine whether DTIC plus BCG is superior to, equal to, or inferior to MeCCNu plus BCG in the post-operative adjuvant therapy of patients with resected Stage I and Stage II malignant melanoma. This study has been completed and closed for Stage I malignant melanoma when no benefit was seen for either regimen over the other or when compared to a controlled untreated group of patients. By contrast, the study is still open for Stage II malignant melanoma where the DTIC plus BCG group is doing better than the MeCCNu plus BCG group. In addition, both treatment groups are doing better than concurrent non-treatment control. We expect Stage II protocol to stay open for another year to accrue a total of 30 patients before closing this protocol. Approximately 15 patients have thus far been treated.

11. ADJUVANT CHEMOTHERAPY AND COMBINATION CHEMOIMMUNOTHERAPY IN THE TREATMENT OF ADVANCED MALIGNANT MELANOMA

Van Echo, D. A., Wiernik, P. H., Aisner, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Medical Oncology Section, *Baltimore, Maryland,* 21201, United States of America

There are only small numbers of patients in all arms of the study but the results are similar to those obtained in larger groups. In the patients receiving DTIC plus MER there is approximately a 29% partial response rate, with responses seen mainly in patients with disease confined to the skin. In the group receiving DTIC alone there is a 25% partial response rate again involving intracutaneous metastases. There is still no evidence that adjuvant chemotherapy delays the time to progressive disease. Accrual has been slow on this study and since large cooperative cancer groups have begun to address this question this study is closed.

12. CANCER CHEMOTHERAPY AND IMMUNOLOGY PROTOCOLS (SWOG PROTOCOLS 7518, 7520, 7580, 7406, 7424/25, 7435, 7475)

Coltman, C. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *San Antonio, Texas,* 78284, United States of America

7518: This study for Stage IIIA and B Hodgkin's disease has had a total of 129 patients entered in the cooperative group and no patients have been entered from San Antonio. The study shows that disease-free and overall survival of the two regimens are essentially equivalent.

7520: No patient entries from this institution on this study. This study has been closed for further patient entry.

7580: This study for the immune evaluation of lymphoma patients in unmaintained remission, patient entries from this institution on the study, is closed for further case accrual.

7406: Five patients entered from this institution and there are a total of 392 cases entered groupwide. This study has been closed for further case accrual. Overall response rate is 69% with the complete response rate from MOPP-Bleo at 64% and the MOP-BAP combinations - 76%.

7424/25: This study employing combination chemoimmunotherapy for patients with disseminated malignant melanoma has no case entries from this hospital. The study is now closed.

7435: No patients have been entered into this study from this hospital. The study is now closed.

7475: One patient has been entered into this study from this institution and the groupwide study is now closed; the manuscript is in preparation.

13. ISOLATION OF TUMOR ANTIGENS OF HUMAN MELANOMA

Bystryn, J., New York University, School of Medicine, Dept. of Dermatology, *550 1st Ave., New York, New York,* 10016, United States of America

The objectives are to continue the purification, isolation and characterization of human melanoma associated antigens (MAA), to study their specificity and the mechanisms of their release by viable tumor cells. If successfully purified, MAA will be used to develop improved assays of humoral immunity to, and circulating antigens associated with, melanoma.

The approach will be to radiolabel macromolecules on the surface of melanoma cells using the lactoperoxidase technique and cytoplasmic proteins with labelled amino acids. Macromolecules will be solubilized by lysis in non-ionic detergents, or if 'shed,' collected in soluble form from culture media. MAA will be identified by double antibody antigen binding assay and radioimmunoassay, utilizing xenogeneic anti-melanoma antibodies exhaustively absorbed with normal tissues. Conventional biochemical procedures and solid phase immunoadsorbents will be used to purify MAA. Specificity will be studied by quantitative absorption, radioimmunoassay and comparison with labelled macromolecules on control cells. If MAA are successfully purified, several aspects of MAA biology relevant to understanding or modification of tumor growth will be studied, including: a) the immunogenicity of MAA in vivo, and methods of enhancing it, b) the mechanisms of release of MAA by viable cells, c) the effect of MAA release on the susceptibility of tumor cells to immune destruction, d) the utilization of purified MAA for the development of improved assays of antibodies to, and circulating antigens associated with, melanoma.

If successful, these studies may provide tumor antigens useful for the specific immunotherapy of melanoma and for assays of the extent, prognosis and response to therapy of this cancer. Furthermore, we hope to understand, and thus influence, antigen release by tumor cells, a process which may have a profound impact on immune resistance to cancer.

14. MAINTENANCE OF HUMAN TUMORS IN VITRO FOR IMMUNOLOGICAL STUDIES

Dent, P. B., Liao, S. K., McCulloch, P. B., McMaster University, Dept. of Pediatrics, *1200 Main St. W., Hamilton, Ontario,* L8S 4L8, Canada

The original objectives of this ongoing project were to validate the use of human cancer cell lines for the study of tumor antigens and immune responses to these antigens using malignant melanoma as the model. To this end, much progress has been made in the development and maintenance of well-defined, mycoplasma-free melanoma lines, and in the search for antitumor immune responses in patients, as well as in the raising of xenogeneic antisera to characterize tumor-associated antigens present on those lines. Continuing work will be directed along the following lines: A) Maintenance of cell stocks and establishment of new cell lines which entails characterization using a number of parameters and, in particular, monitoring for mycoplasma. B) Development of highly antigenic sublines. Xenogeneic antibodies have revealed that some cell lines are consistently more reactive than others suggesting that melanoma associated antigens (MAAs) may be expressed to a different degree on different cell lines. Highly reactive clones will be isolated, and after determining whether this high reactivity is stable we will evolve a panel of such cell lines to define the spectrum of MAA polymorphism as revealed by xenoantibody. This panel will then be evaluated in immunological tests using human sera to determine if the same MAAs are recognized by and are relevant to man. C) Use of selected tumor cells for immunotherapy of melanoma. The use of specific active immunotherapy in melanoma has not been adequately evaluated in terms of potential benefit and in terms of the clinical relevance of the antitumor immune responses which can be measured in such patients. We plan to carry out a preliminary nonrandomized trial of patients with recurrent melanoma to determine if the induction of specific antimelanoma antibodies can be correlated with an improved survival.

15. CELL-MEDIATED IMMUNITY AND TRANSFER FACTOR IN HUMAN MALIGNANT MELANOMA

Valentine, F. T., New York University, School of Medicine, Dept. of Medicine, *550 1st Ave., New York, New York,* 10016, United States of America

This is part of a broader project. A summary of this subproject is not available.

16. IMMUNE RESPONSIVENESS AND IMMUNOTHERAPY FOR MELANOMA

Seigler, H. F., Duke University, School of Medicine, Dept. of Medicine, *Box 3711, Durham, North Carolina,* 27710, United States of America

This is part of a broader project. A summary of this subproject is not available.

17. IMMUNOTHERAPY OF MALIGNANT MELANOMA (HUMAN)

Wright, P. W., University of Washington, School of Medicine, Dept. of Medicine, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington*, 98195, United States of America

This is part of a broader project. A summary of this subproject is not available.

B. IMMUNOTHERAPY OF LEUKEMIAS, LYMPHOMAS, SARCOMAS, AND MYELOMAS

18. ACUTE MYELOID LEUKEMIA - IMMUNOTHERAPY WITH BCG AND IRRADIATED ALLOGENEIC AML CELLS VERSUS NO MAINTENANCE THERAPY (ABBREV)

Harris, R., Freeman, C. B., Zuhrie, S. R., Delamore, I., University of London, St. Mary's Hospital Medical School, Dept. of Medical Genetics, *Norfolk Pl., London W2 1PG, England, United Kingdom*

OBJECTIVE: To determine whether immunotherapy alone prolongs first remission length, increases reinduction rate and lengthens post-relapse survival.

APPROACH: Patients achieving complete remission with daunorubicin and cytosine and receiving six week consolidation with cyclophosphamide and 6-thioguanine are randomized to receive either immunotherapy alone with 10 to the 9th power irradiated allogeneic AML cells plus BCG or no further maintenance treatment.

PROGRESS: The median first remission length for 21 patients receiving immunostimulation is 35 weeks versus 21 weeks for 20 patients on no maintenance treatment which is significant (P less than 0.05).

19. USE OF BONE MARROW TRANSPLANTS IN THE TREATMENT OF ACUTE LEUKEMIA

Kay, H. E., Powles, R. P., Barrett, A. J., Royal Marsden Hospital, Leukaemia Unit, *Downs Rd., Sutton SM2 5PT Surrey, United Kingdom*

OBJECTIVE: To explore the value of bone marrow transplantation in the treatment of acute leukemia, especially acute myeloid leukemia in first remission.

APPROACH: The protocol is that used in Seattle, i.e., high dose cyclophosphamide and whole-body irradiation but with two main differences: a) the dose-rate of irradiation will be slow (2.5 rad/min) and unfractionated; b) alternative means to prevent GvH reactions are being explored.

PROGRESS: The program is continuing although Dr. Barrett, the L.R.F. fellow, has left. Early results indicate that BM transplants are well-tolerated by patients in first remission. Of 11 with AML in first remission there has been one relapse (an identical twin), two fatal GvH reactions, and eight continue in remission at periods of four weeks to 18 months; mild or chronic GvH is present in two.

20. AML IMMUNOTHERAPY AND ITS EFFECT ON SURVIVAL, REMISSION, IMMUNITY TO LEUKEMIA CELLS, AND COLONY STIMULATING ACTIVITY (ABBREV)

Reizenstein, P., Hast, R., Lantz, B., Anderson, B., Beran, M., Uden, A., Karolinska Sjukhuset, Division of Hematology, *S10401 Stockholm, Sweden*

OBJECTIVE: To confirm our earlier findings on efficacy of immunotherapy in accute myeloid leukemia (AML), and to identify its mechanism of action.

APPROACH: Leukemia patients in remission given chemotherapy plus immunotherapy (CIT) are compared to patients given only chemotherapy (CT). CIT with BCG is compared to CIT with BCG plus leukemia cells. Leukemia cells are characterized by measuring antigenicity, cloning capacity, peroxidase and terminal transferase content, Ia-antigens, C3 and Fc-receptors, and soluble cell surface antigens. Non-specific immunocompetence and specific immunity to leukemia cells are measured in vitro and in vivo. Cellular and immune characteristics are correlated to prognosis. Macrocyte function, including CSA product, is monitored.

PROGRESS: Survival, frequency of second remissions, and, surprisingly, remission duration are significantly increased in the CIT group. Non-specific immunocompetence is not higher in the CIT than in the CT group; in remission both are normal. Spontaneous 'immunity' to leukemic cells (in vitro) and proliferation of autologous lymphocytes are found in 1/4 of patients, but these patients do not have a better prognosis than remaining 3/4. Nor is frequency or intensity of 'immunity' to leukemia cells increased by CIT. Nor could we show that increased phagocytic or fungicidal function of macrophages, or fewer infections during relapse, explained IT-effect. According to a preliminary study, stem-cell number and function in bone marrow during remission are also normal in both the CIT and the CT groups. Our future aim is to determine if IT stimulates CSA. This could explain longer remission duration and more second remissions in the CIT group.

21. CHEMOTHERAPY AND CHEMOIMMUNOTHERAPY OF CANCER

Holland, J. F., City University of New York, Mount Sinai School of Medicine, Dept. of Neoplastic Diseases, *5th Ave. at E. 100th St., New York, New York*, 10029, United States of America

In the last calendar year, Program Project Grant (CA15936-05) has been vigorously pursued by the investigators of the Mount Sinai School of Medicine under the Principal Investigatorship of James F. Holland, M.D.

Chemoimmunotherapy of acute myelocytic leukemia continues to provide exciting data.

Research in lung cancer has involved the investigation of a 4-drug combination regimen, MACC plus levamisole, and MACC plus C. parvum.

Our investigation of 4-drug second line chemotherapeutic responses in disseminated breast cancer continues to be productive.

Adriamycin and platinum continue to be the central building blocks for chemotherapeutic regimens in patients with ovarian cancer.

A continuing assessment of our chemotherapeutic regimens in prostatic cancer identifies 15 of 19 responders, with six of eight with measurable lesions showing 6% or greater regression of the disease. Remaining responders were all characterized by major reduction in pain and reduction in alkaline and acid phosphatase.

Gastrointestinal cancer investigations continue with a central attention to fluorouracil by continuous infusion and intermittent intravenous bolus dosing with mitomycin-C and oral treatment with hexylmethylmelanamine.

Experimental chemotherapy has included active clinical investigations of several new drugs, m-AMSA, maytansine, dichloromethotrexate, L-phosphonoacetyl-l-aspartate (PALA), indocine-n-oxide, and L-alanosine.

In the microbiological research, serum opsonins as the determinant of success in granulocyte transfusions are still being studied.

Analytical biochemistry of opportunistic infections has made major progress in identification of D-arabinitol, a metabolite of Candida albicans which can be reproducibly quantified in human serum.

BIBLIOGRAPHIC REFERENCES: Takahashi, I., Ohnuma, T., and Holland, J.F.: Comparison of biological effects of dichloromethotrexate and methotrexate on human leukemia cells in culture. Cancer Res. 39:1264-1268, 1979. Ambinder, E.P., Perloff, M., Ohnuma, T., Biller, H.F., and Holland, J.F.: High dose methotrexate followed by citrovorum factor reversal in patients with advanced cancer. Cancer 43:1177-1182, 1979.

22. BONE MARROW COLONY FORMING UNITS IN PATIENTS WITH ACUTE LYMPHATIC LEUKEMIA ON BCG AND CHEMOTHERAPY OR ON CHEMOTHERAPY ONLY (ABBREV)

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OBJECTIVE: To compare the number of colony forming units in bone marrow of patients with acute leukemia in remission, in those patients given immunotherapy with BCG, with those treated similarly and not given BCG.

APPROACH: Once remission is achieved in patients with ALL and CNS proplylaxis has been completed, intermittent

maintenance chemotherapy will be begun with a six week cycle of chemotherapy followed by two weeks of no therapy. Patients will be randomized to receive or not receive BCG in the middle of the 2 week period of no therapy.

The agar culture technique of Pike and Robinson will be used. Bone marrow will be obtained at the end of the six week period of chemotherapy, on the day of BCG administration (in both groups) and on the day chemotherapy is due to resume. Comparison will be made between the number of colonies produced in both groups. The study is an attempt to assess the effect of BCG in committed granulocyte-progenitor stem cells in a group of patients who otherwise receive identical treatment.

23. CHEMOIMMUNOTHERAPY (BCG) CONTROLLED TRIAL IN ACUTE LYMPHOCYTIC LEUKEMIA OF CHILDHOOD

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OBJECTIVES: 1. To investigate whether intermittent chemotherapy, combined with immunotherapy, prolongs the duration of the first complete remission, compared with intermittent chemotherapy alone. 2. To investigate whether intermittent chemotherapy, combined with immunotherapy, results in longer survival than intermittent chemotherapy alone. 3. To determine the effects of these regimens on immunological function and on the incidence of infective and other complications.

APPROACH: A cooperative study (Australian Cancer Society Childhood Leukemia Study Group) using a standard protocol. Chairman of Study Group is Dr. H. Ekert, Royal Children's Hospital, Parkville, Victoria, 3052.

24. IN VITRO IMMUNIZATION TO HUMAN TUMOR CELLS

Sharma, B. S., University of California, Irvine Campus, California College of Medicine, Dept. of Medicine, *Irvine, California*, 92664, United States of America

Recently, we have demonstrated that remission lymphocytes from acute lymphoblastic leukemia (ALL) patients are able to become cytotoxic to their respective leukemic blasts (ALB) following exposure to ALB, soluble microbial extracts, poly AU, levamisole, or cyclophosphamide. However, there seems to be a variation in the ability of ALB to stimulate and the ability of patients' lymphocytes to become cytotoxic. In some cases, when ALB alone could not generate cytotoxic lymphocytes the combination of ALB and BCG extract induced greater cytotoxicity than was induced by BCG extract alone. At present, the mechanisms of nonresponsiveness of certain ALL patients' lymphocytes, and efforts to correct and/or potentiate the responsiveness to tumor cells is being investigated. The presence of suppressor cells, lack of helper cell activity, production of prostaglandins, and/or poor immunogenicity of leukemia cells are under investigation as a possible cause of nonresponsiveness of variation in the response of lymphocytes to leukemia cells. The adherent suppressor cells will be removed by either glass wool column or nylon wool column. Lymphocytes will be fractionated on discontinuous gradient of bovine serum albumin. Tr, Tmu, and Tphi subpopulations of T cells will be separated and their abilities to become cytotoxic or suppress the induction of cytotoxicity will be tested. Effect of thymosin, levamisole, poly AU or cyclophosphamide will be determined on nonresponder lymphocytes. Whether prostaglandins are responsible for the inability of some leukemia cells to stimulate lymphocytes will be studied. Attempts will be made to see whether indomethacin (an inhibitor of prostaglandin synthesis) will restore the ability of remission lymphocytes to become cytotoxic against leukemia cells. The inability of certain leukemia cells to sensitize lymphocytes may be due to poor antigenicity of leukemia cells. Attempts will be made to modify leukemia cells by chemicals (e.g. iodoacetamide) or enzymes (e.g. Vibrio cholerae neuraminidase) and then their ability to stimulate lymphocytes to become cytotoxic will be determined.

25. ANTIGEN-ANTIBODY INTERACTIONS IN NEOPLASTIC DISEASE

Minden, P., Farr, R. S., Kelleher, P. J., National Jewish Hospital & Research Center, Dept. of Medicine, *3800 E. Colfax Ave., Denver, Colorado*, 80206, United States of America

The use of cellular immunoadsorbents to prepare antibody that distinguishes between syngeneic surface antigens on two guinea pig hepatocarcinomas: Xenogeneic antibodies with reactivity for surface determinants of guinea pig line-10 hepatocarcinoma cells were isolated using cellular immunoadsorbents prepared by coupling formalin treated line-10 cells to DE-52. Antibodies prepared in this manner exhibited a high degree of reactivity for line-10 surface determinants. They also reacted to some extent with surface determination of syngeneic line-1 cells. Further restriction of antibody reactivity was accomplished by passing the antibodies through cellular immunoadsorbents prepared with line-1 cells. By direct binding studies, these antibodies showed significantly reduced activity for line-1 cells and no reactivity for guinea pig spleen cells or for unrelated murine EL-4 lymphoma cells. By a competitive radioimmunoassay these antibodies reacted only with determinants expressed on the surfaces of line-10 cells and not on line-1, L2C, spleen, thymus or EL-4 cell surfaces. The sequential use of immunoadsorbents made of antigenically distinct syngeneic tumor cells made it possible to prepare antibodies with restricted reactivity for line-10 associated antigens and is an additional approach by which 'tumor-specific' antibodies can be prepared.

Immune complexes (IC) in children with leukemia: Relationship to disease characteristics and antibody response to BCG in patients receiving BCG immunotherapy. Determinations were made in sera obtained at three-month intervals from individual children with leukemias over a two-year period. At time of diagnosis, IC were found in sera from 6 of 41 patients with acute lymphoblastic leukemia (ALL). Three of 6 patients with T cell leukemia had IC at diagnosis, as compared to 3 of 55 with null cell leukemia. Of the patients followed for 6 months or more, 34% had IC during therapy and the incidence of relapse was unrelated to the presence or absence of IC. The presence of IC by themselves did not portend either a favorable or an unfavorable prognosis. Sera taken at the time of diagnosis from children with acute non-lymphoblastic leukemia had a higher incidence, 36% with IC, than sera from ALL patients (15%). Antibodies in sera from ALL patients at diagnosis had a lower capacity to bind a BCG antigen than sera from normal controls. (Text Abridged.)

BIBLIOGRAPHIC REFERENCES: Carr, R.I., Chakraborty, A.K., Brunda, M.J., Davidson, P.T., Damle, P.B., Hardtke, M.A., Gilbride, K.J., and Minden, P.: Immune complexes and antibodies to BCG in sera from patients with mycobacterial infections. Clinical Exp. Immunol., 39:562-569, 1980. Mathews, H.L., Brunda, M.J., and Minden, P.: The use of cellular immunoadsorbents to prepare antibody that distinguishes between syngeneic surface antigens on two guinea pig hepatocarcinomas. J. Immunol. 124:1141-1147, 1980.

26. BONE MARROW TRANSPLANTATION IN CHILDREN WITH LEUKEMIA

Dooren, L. J., Vossen, J. M., Rothbarth, P. H., deKoning, J., Kamphuis, R. P., Academisch Ziekenhuis Leyden, Dept. of Pediatrics, *Rijnsburgerweg 10, 2333 AA Leyden, Netherlands*

OBJECTIVE: Treatment of children with acute leukemia with poor prognosis in first remission by transplantation of bone marrow cells from MHC identical sibling donors as compared with conventional cytostatic therapy.

APPROACH: 1. Selection of treatment group for high risk leukemia patients. 2. Optimization of cell reductive and transplantation treatment. 3. Prevention and treatment of complications; optimalization of supportive treatment, including psychosocial guidance. 4. Longitudinal follow-up of several aspects of humoral and cellular immune capacities following bone marrow transplantation.

PROGRESS: Project will start approximately September 1, 1979.

27. EFFECTS OF THERAPY ON TUMOR-SPECIFIC IMMUNITY

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We propose to study the effects of chemotherapy and immunotherapy on immunity to autochthonous or allogeneic tumor cells ('tumor-specific' immunity) in patients with a solid tumor or acute leukemia. A large proportion of the tumor cells

obtained by biopsy will be stored in DMSO at minus 70 degrees, to permit replacement of tissue cultures after 3 month continuous passage to minimize nonspecific lysis. Blood mononuclear cells and serum will be obtained weekly or biweekly from each patient before, during and after therapy in studies ranging from 6 weeks to 2 years. Clinical response to therapy will be measured objectively. Cell-mediated cytotoxicity will be measured by a 48-hour assay in 16 mm in diameter plastic wells, with 3H-proline or 125IUdR-labeled tumor cells. Blocking factor will be estimated by preincubating serum and tumor cells before adding mononuclear cells. Cytophilic antibodies will be titrated by the arming of mouse monocytes to form rosettes with tumor cells, while related antibody sensitizing for antibody-dependent cellular cytotoxicity (ADCC) will be titrated by the arming of neutral human monocytes to lyse tumor cells. 'Nonspecific' cytotoxicity will be studied by examining stratified populations of 'normal' individuals for reactivity, and by determining whether continuously cultivated tumor cells become overly sensitive to lysis. It will be established which subpopulations of mononuclear cells are the major cytotoxic effectors and their specificity will be determined. Lymph node cells of melanoma patients receiving intralymphatic immunotherapy with MER will be compared with blood lymphocytes. Patients with acute myelocytic leukemia given immunotherapy will be studied to discern whether humoral immunity to the leukemia is improved. We hope to determine whether our current regimens of tumoricidal therapy are suppressive towards tumor-specific immunity, and whether putative immunotherapeutic regimens are stimulatory.

BIBLIOGRAPHIC REFERENCES: Rao, V.S., Grodzicki, R.L., and Mitchell, M.S. Specific in vivo inhibition of macrophage receptors for cytophilic antibody by immune complexes. Cancer Res. 39: 174-182, 1979. Bennett, J.A., and Mitchell, M.S. Induction of suppressor cells by intravenous administration of Bacillus Calmette-Guerin and its modulation by cyclophosphamide. Biochem. Pharmacol. (Arnold Welch Festschrift) 1979, in press.

28. OMRF 74-02 - ACUTE NON-LYMPHOCYTIC LEUKEMIA (A-CAT)

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OBJECTIVES: To compare induction efficacy of (1) adriamycin plus OAP; (2) adriamycin plus Ara-C; (3) cyclophosphamide, adriamycin, Ara-C and thioguanine, in adults with acute non-lymphocytic leukemia. The best regimen will be the one which is most efficient in clearing the bone marrow of leukemic cells during the first and second courses, thus improving the remission rate. To compare the effectiveness of 5-day maintenance - (1) OAP plus BCG; (2) Ara-C plus BCG; (3) Ara-C, thioguanine and BCG - in prolonging the duration of complete remission.

REMISSION INDUCTION: Eligible patients will be randomized to one of three treatment limbs, as follows: LIMB I: Adriamycin plus OAP; LIMB II: Adriamycin plus Ara-C; LIMB III: Adriamycin, Cytoxan, Ara-C and thioguanine. A bone marrow aspiration (or a biopsy if a dry tap is obtained) will be carried out on day 18 to determine whether the bone marrow is cleared of leukemic cells and if marrow hypoplasia has been reversed, so that a further course can be instituted.

REMISSION CONSOLIDATION: Three consolidation courses will be administered at 14-day intervals, as follows: LIMB I: Ara-C - 100 mg/m2/day for 5 days by constant IV infusion; prednisone - 50 mg b.i.d., PO for 5 days; vincristine - 1 mg IV on day 1. LIMB II: Ara-C - 100 mg/m2 day for 5 days, by constant IV infusion. LIMB III: Ara-C - 100 mg/m2/day for 5 days, by constant IV infusion; thioguanine - 2 mg/kg/day, PO, for 5 days. Dosage adjustments in Ara-C may be made when marrow hypoplasia occurs; the dose of subsequent courses can be reduced by 10%.

There is one veteran patient on this study at this time. This study is now closed to new patient entry.

29. OMRF 7505 - CHRONIC GRANULOCYTIC LEUKEMIA

Bottomley, R. H., Murray, J. L., Grozea, P. N., Hussein, K. K., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Hematology Section, *921 N.E. 13th St., Oklahoma City, Oklahoma,* 73104, United States of America

OBJECTIVES: To determine if the combined addition of splenectomy and immunotherapy with BCG to intermittent busulfan therapy a) delays the onset or reduces the incidence of blastic transformation, and b) improves the survival of previously untreated patients with chronic granulocytic leukemia, utilizing the results of SWOG study no. 545/546 as a historical control.

PLAN: The patient will take busulfan until the leukocyte count is between 5,000 and 9,000. One to three weeks after busulfan has been discontinued, the spleen will be removed if medically feasible. Two to three weeks after removal of the spleen, the patient will be treated with busulfan alternating with BCG scarification. When the white blood count is greater than 40,000, the busulfan will be taken by mouth daily until the white blood count is 9,000 or less; then, BCG will be given by scarification at weekly intervals until the white blood count is greater than 40,000. At this time, busulfan therapy will be resumed. This treatment will continue as long as the disease is responding, providing the patient has recovered from the side effects of the previous treatment.

This study is closed to new patient entry, with one V.A. patient remaining on the study.

30. SOUTHWEST ONCOLOGY GROUP PROTOCOLS 7525, 7521, 7511

Coltman, C. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *San Antonio, Texas,* 78284, United States of America

SWOG 7525 - This study is to determine the effect of BCG in multiple myeloma patients who are responding to and will continue to receive vincristine, melphalan, cyclophosphamide and prednisone and to evaluate immunocompetence of these patients before and during therapy. No patients were entered into this study. SWOG 7521 - To determine the ability of BCNU, hydroxyurea, and imidazole carboxamide to prolong disease free interval and survival among patients with surgically treated primary malignant melanoma who are at high risk of recurrent disease. Also to determine whether BCG can augment this effect and to evaluate the immune competence of these patients before and during therapy. Four patients were entered into this study which began in November of 1975 for Class I new patients. There is no indication that chemotherapy is of any value in Class II patients. There is statistical evidence that patients undergoing chemoimmunotherapy do significantly less well than those undergoing chemotherapy alone (P equals 0.007.) SWOG 7511 - To determine the effect of BCG in multiple myeloma patients who are responding to and will continue to receive vincristine, melphalan, cyclophosphamide and prednisone, and to evaluate immunocompetence of these patients before and during therapy. The study began in September 1975 and closed in March of 1977. No patients were entered into the study. There is no statistical difference in survival in the various limbs of this study.

31. CONTROLLED CLINICAL TRIAL (PHASE III) OF CHEMOIMMUNOTHERAPY IN CHILDHOOD ACUTE LYMPHOCYTIC LEUKEMIA (ALL)

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OBJECTIVES: 1) Compare duration of first complete remission maintained with intermittent chemotherapy and BCG compared with intermittent chemotherapy only. 2) Determine the effects of these regimens on immunologic function and infective complications.

HISTOLOGICAL TYPE AND CLINICAL STAGE: Children with morphologic and cytochemical diagnosis of ALL are stratified on the basis of age, organomegaly and total white cell count into good prognosis (WCC 5-1000), average prognosis (WCC 10-30,000) and intensive therapy (WCC 30,000) groups.

TREATMENT MODALITY AND PROTOCOL OUTLINE: Good and average prognosis induced with vincristine and prednisolone, but cytosine and asparaginase are added in average prognosis groups. Intensive therapy group induced with cytosine cyclophosphamide and asparaginase. All children given

prophylactic skull irradiation (2400r) and 4 injections of intrathecal methotrexate. Remission is confirmed by bone marrow aspiration. All patients are then randomized into either group A - 6 weeks of chemotherapy consisting of 6MP daily, weekly methotrexate and monthly vincristine followed by 2 weeks of no treatment; or group - some chemotherapy but one week after its cessation 0.25ml of BCG is inoculated with a Heaf gun. (BCG Commonwealth Serum Laboratory Pasteur strain protein content 75mg/ml viable organisms 6-20 x 10 to the 6th power/mg).

PRESENT STATUS: Ninety-seven randomized with at least 3/12 of follow up; 52 pending analysis. 63% C.R. at 152 weeks. No significant difference in BCG arm (48 patients) vs. no BCG arm (49 patients).

32. SEQUENTIAL THERAPY FOR INDUCTION AND MAINTENANCE OF REMISSION IN ACUTE MYELOBLASTIC LEUKEMIA

Pavlovsky, S., EppingerHelft, M., Grupo Argentino de Tratamiento de la Leucemia Aguda, *P. de Melo 3081, 1425 Buenos Aires, Argentina*

This is a Phase III study with the following objectives: 1) To confirm the usefulness of sequential induction treatment with daunorubicin, vincristine, prednisone followed by cytosine arabinoside-6-mercaptopurine in a new series of patients. 2) To compare the effectiveness of an intensive and intermittent maintenance treatment alternating monthly courses of cytosine arabinoside-6-mercaptopurine-CCNU with daunorubicin-vincristine-prednisone with or without adjuvant immunotherapy. 3) To study the effectiveness of prophylactic CNS leukemia treatment with 5 doses of intrathecal methotrexate early in remission.

THERAPEUTIC REGIMEN: INDUCTION: Daunorubicin (DNR) 1 mg/kg i.v. and vincristine (VCR) 1.5 mg/sq m i.v., both drugs at weekly intervals for 3 doses associated with prednisone (PRED) 40 mg/sq m daily orally for 14 days. Then: Cytosine arabinoside (C.Ara) 100 mg/sq m day i.v. plus 6-mercaptopurine (6MP) 2.5 mg/kg/day orally for 5 days until complete remission is achieved.

MAINTENANCE: C.Ara 100 mg/sq.m i.v. for 5 days plus 6-MP 2.5 mg/kg/day orally for 5 days plus CCNU 75 mg/sq m orally only once, alternating monthly with DNR 1 mg/kg/day i.v. plus VCR 1.5 mg/sq m/day, both drugs only for one dose, and PRED 40 mg/sq m/daily orally for 7 days.

PROPHYLACTIC CNS LEUKEMIA TREATMENT: During the first 5 weeks of maintenance the patients also receive intrathecal methotrexate (MTX) (12 mg/sq m) at weekly intervals.

IMMUNOTHERAPY: Half of the patients receive Corynebacterium parvum 4 mg (2 ml.) subcutaneously once a week between the maintenance courses.

PROGRESS: Out of 181 evaluable cases, 80 (44%) achieved complete remission. A total of 36 out of 45 randomized to chemotherapy relapsed, compared to 32 out of 35 of the chemoimmunotherapy group. Median duration of complete remission and survival was: for the chemotherapy group 8 and 15 months, for the chemoimmunotherapy group 5 and 10 months. This difference is not significant. Six patients (7%) had CNS leukemia at diagnosis. None had CNS leukemia relapse after prevention and IT methotrexate.

A progress report of this study was presented at the 14th ASCO meeting (page 402) 1978.

33. CANCER AND LEUKEMIA GROUP B

Leventhal, B. G., Johns Hopkins University, School of Medicine, Dept. of Pediatrics, *725 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

We plan to continue our active participation in CALGB protocols. Patient accrual will continue at least at the present level and may well increase as the activity of the pediatric subcommittee increases and more pediatric protocols are activated by the group as a whole. Dr. Kaizer is involved in planning a new non-Hodgkin's lymphoma protocol for the group.

The immunotherapy subcommittee is exploring possible agents other than intradermal MER for immunotherapy and new protocols will soon be emerging.

34. STUDY OF THE POSSIBLE PREVENTIVE EFFECT OF BCG AGAINST CANCER IN ADULTS

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Studies have shown that BCG may have a non-specific preventive effect against mortality due to leukemia in children aged 0 to 4 years. This cohort study aims to determine if BCG could have a preventive effect against cancer in adults by comparing mortality and morbidity from cancer of all types among vaccinated and unvaccinated nurses. Names and addresses of all nurses graduated in the province of Quebec between 1952 and 1967 were obtained. A questionnaire was sent to each. This questionnaire included questions on age, date of graduation, marital status, BCG vaccination status, names of diseases of all kinds diagnosed since graduation, date of the event, place of diagnosis and treatment, name of physician, smoking habits, number of pregnancies and abortions and finally name and cause of death of classmates known to have deceased since graduation. The BCG vaccination status was verified in the central BCG record of l'Institut Armand-Frappier. Information has been received from 12,856 nurses of a possible total of 21,000, and 453 deaths have been reported. These data will be analyzed, taking into consideration vaccination status, age and time since vaccination.

35. BCG VACCINATION AND LEUKEMIA

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The possibility that the reported non-specific immunity conferred by BCG may have some influence in the prevention of leukemia in children is being investigated. In Israel a unique opportunity presents itself for studying the incidence of leukemia in BCG vaccinated children and in a non-vaccinated control group: since 1955 routine BCG vaccination of newborns has been carried out in all maternity wards in Israel - with the exception of the Jerusalem hospitals, where it was never introduced. The incidence of leukemia in children born in Israel since 1955, diagnosed after 1960 (the year the Israel Cancer Registry was founded) and up to the age of ten will be studied. Two hundred and sixty-three cases of leukemia in children have been identified. Their places of birth are now being ascertained.

36. TRANSFER FACTOR THERAPY IN LEUKEMIA AND IMMUNE DEFICIENCY DISEASES

Lampkin, B. C., Childrens Hospital Medical Center, *3245 Burnet Ave., Cincinnati, Ohio,* 45229, United States of America

This is part of a broader project. A summary of this subproject is not available.

37. IMMUNOTHERAPY STUDIES - LEUKEMIA (HUMAN)

Henderson, E. S., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York,* 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

38. T LYMPHOCYTES IN LYMPHOMA PATIENTS DETERMINED BY QUANTITATIVE AND SCORING EVALUATION OF ROSETTE FORMATION

Jamra, M., Pozzi, D., Dorlhiac, P., Universidade de Sao Paulo, Faculdade de Medicina, Dept. of Hematologia, *Ave. Dr. Arnaldo 455, Sao Paulo 01246, Brazil*

OBJECTIVES: Study of receptors for sheep red blood cells (SRBC) present on T lymphocytes in lymphomas, principally in Hodgkin's disease (H) patients, who are known to have impaired lymphocyte function. Studies made by Khan et al. (Cancer Res. Proc. 16:65,75) show a modification of T lymphocytes after in vitro treatment with transfer factor. In our study, we adopted the scoring technique for quantitative evaluation of T lymphocyte receptors in untreated H and non-Hodgkin's (n-H) lymphomas, during and after radio- and/or chemotherapy.

APPROACH: Blood was collected from normal donors and lymphoma patients and lymphocytes separated on ficoll-hypaque gradient. Lymphocytes washed with isotonic saline solution were resuspended in Hank's solution to obtain a 4 times 10 to the 6th power/ml concentration. SRBC washed twice with isotonic saline solution were resuspended in Hank's solution at 8 times 10 to the 7th power/ml concentration. Transfer

factor (TF), prepared from peripheral lymphocytes, one unit (1 ml) corresponding to 10 to the 8th power lymphocytes, was diluted 1:50 before use. Lymphocyte and SRBC suspensions, 0.25 ml of each, were mixed and incubated for 15 min. at 37 degrees C, centrifuged and left overnight at 4 degrees C. Effect of TF was evaluated by adding 0.025 ml of 1:50 diluted TF to the lymphocyte suspension just before addition of SRBC and incubation. All samples were evaluated by counting percentages and by scoring. For scoring, the SRBC attached to each lymphocyte were counted, using the following criteria: SRBC 0-2, 3-5, 6-8, 9-11, 12 or more corresponding to 0, 1 plus, 2 plus, 3 plus and 4 plus, respectively.

RESULTS: Ten normal donors show no statistically significant differences in percentages and scores either before (59.5% plus or minus 4.8/127.9 plus or minus 8.87) or after addition of TF (64.0% plus or minus 5.5/142.9 plus or minus 13.0).H patients' (10 cases) lymphocytes resulted in 44.1% plus or minus 9.5/79.8 plus or minus 18.56 before and 59.6% plus or minus 7.77/112.6 plus or minus 18.65 after addition of TF, the differences being statistically highly significant (p less than 0.01). (Text Abridged.)

39. IMMUNOLOGICAL STUDIES AND IMMUNOTHERAPY PILOT TRIAL IN HODGKIN'S DISEASE

Grifoni, V., Tognella, S., Pierri, I., Sangiuolo, A., Scarsi, P. G., Piccardo, C., Scudeletti, M., Universita degli Studi di Genova, Cattedra Clinica Medica, *Viale Benedetto 15-2, 116132 Genoa, Italy*

OBJECTIVES: (a) To further characterize the lymphocytotoxic autoantibody present in the sera of patients with Hodgkin's disease. To identify cell subpopulations which produce it, and target cell and subcellular fractions. To study interference of levamisole with the action of such antibody. (b) To explore various modalities of intervention in order to correct the immunodeficiency of such patients, and possibly to influence the course of the fundamental disease itself.

APPROACH: (a) Lymphoid cells from peripheral blood, lymph nodes, and spleens of patients with Hodgkin's disease will be separated through Ficoll isokinetic gradient in order to identify source-cell of and target-cell for the autoantibody. Lymphoid cell lysate and membrane will be submitted to fractionation employing disc electrophoresis and Sephadex G-200 gel filtration in order to purify the antigen(s) involved. Interference of levamisole in the in vitro reaction between the cytotoxic antibody and lymphocytes will be studied. (b) Lymphocytapheresis, autoantibody elution, and lymphocyte reinfusion should be done in subjects in which marked autocytotoxicity is present. Allogeneic lymphocyte transfusion and treatment with immunomodulator drugs should also be exploited.

PROGRESS: (a) Evidence has been produced that levamisole is capable of preventing and partially reversing the inhibition of E-rosette formation induced by Hodgkin's cytotoxic serum (Del Giacco, S., Tognella, S., Leone, A.L., Locci, F., Cornaglia, P., Sangiuolo, A., Grifoni, V.: Interference of levamisole with inhibition of E-rosette formation by Hodgkin's disease and systemic lupus eryhthematosus cytotoxic sera: Blood 53, 1002-1006, 1979).

40. NO. 7552 - COMBINATION CHEMOTHERAPY AND IMMUNOTHERAPY FOR PREVIOUSLY TREATED STAGE IIIB AND IV HODGKIN'S DISEASE

Spaulding, M. B., Sanani, S., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *3495 Bailey Ave., Buffalo, New York*, 14215, United States of America

This study compares two combinations of chemotherapy, one being the standard CCNU, Velban, procarbazine, prednisone combination and the other a noncrossresistant combination of bleomycin, adriamycin, vincristine and streptozotocin with alternating courses of these eight drugs in patients who have relapsed from previously treated Hodgkin's disease. We have entered no patients from this VA Medical Center. This study is part of a larger Cancer and Acute Leukemia Group B study and is still under way.

41. SOUTHWEST ONCOLOGY GROUP PROTOCOL 7808, COMBINATION MODALITY TREATMENT FOR STAGE III AND IV HODGKIN'S DISEASE, MOPP NO. 6

Coltman, C. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *San Antonio, Texas*, 78284, United States of America

This study is designed to attempt to increase the complete remission rate induced with MOP-BAP alone utilizing involved field radiotherapy in patients with Stage III and IV Hodgkin's disease achieving a PR at the end of 6 cycles of MOP-BAP, and to determine if immunotherapy maintenance with levamisole or consolidation with low dose involved field radiotherapy will produce significantly longer remission durations over a no-further-treatment group when CR has been induced with 6 cycles of MOP-BAP in Stage III and IV Hodgkin's disease.

One patient has been entered into this study and thus far a total of 64 patients have been entered into the groupwide study which is continuing.

42. THE IMMUNOTHERAPY OF ANIMAL AND HUMAN SARCOMAS

Rosenberg, S. A., Strausser, J., Lotze, M., Almaraz, R., Eberlein, T., Rosenstein, M., Brown, J., Yron, I., Schwarz, S., Wexler, H., Spiess, P., Hyatt, C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Surgery Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Detailed studies of tumor-host immune interactions in animals and humans with sarcomas are being performed in an attempt to develop new immunodiagnostic and immunotherapeutic techniques for diagnosis and treatment of these tumors. Immune response to murine sarcomas has been extensively evaluated and both tumor-specific and fetal antigens have been identified. Fetal antigens have been identified on human osteogenic sarcomas and these antigens are now being characterized. Attempts are being made to develop adoptive immunotherapeutic techniques utilizing transfer of cells from xenogeneic species to humans with cancer. Techniques for the prolonged growth of cytotoxic T cells have been developed, and attempts to use these cells for adoptive immunotherapy are in progress.

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43. CALGB PROTOCOL 7681 - INVESTIGATION OF THE EFFECTS OF ADRIAMYCIN WITH AND WITHOUT ADDED MER IN SOFT TISSUE SARCOMAS

Spaulding, M. B., Sanani, S., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *3495 Bailey Ave., Buffalo, New York*, 14215, United States of America

This study was designed to evaluate two dose schedules of adriamycin, with and without the use of an immunostimulant, in soft tissue sarcoma which was metastatic. Once patient was treated at this institution without problems and without a response. The project is not closed. This study is a part of a larger study and no results are yet available.

44. INTERFERON THERAPY OF MYELOMATOSIS AND OTHER TUMOR DISEASES

Strander, H., Radiumhemmet, *S10401 Stockholm, Sweden*

OBJECTIVES: A) In a prospective randomized study, comparison is made between intermittent melphalan-prednisolone treatment and interferon therapy against myelomatosis. B) In a pilot study different doses are compared in the treatment of juvenile laryngeal papillomatosis with interferon therapy. C) Pilot studies with interferon therapy are being done on other tumor diseases.

APPROACH: A) Myelomatosis - the medical departments of Huddinge Hospital, Sodersjukhuset, Serafimerlasarettet, Akademiska sjukhuset in Uppsala, Regionsjukhuset in Orebro and Radiumhemmet at the Karolinska Hospital are taking part in a prospective randomized investigation where intermittent melphalan-prednisolone treatment is compared with interferon ther-

apy. If the inteferon therapy arm compares unfavorably with the arm treated with chemotherapy, discussions will take place in order to try increased interferon dosaging and combination therapy with melphalan and interferon. Earlier it was shown at Radiumhemmet that interferon therapy can have an effect on myelomatosis. B) Juvenile laryngeal papillomatosis - a pilot study is being done in cooperation with the ENT section at the Karolinska Hospital. Seven patients treated with interferon have all shown regression. After cessation of interferon therapy a new progression of the disease is revealed. Therefore, a pilot study is being made in order to determine how much interferon is required for these patients in order to prevent recurrence. C) Other tumor diseases - interferon is also being given to patients suffering from oat-cell cancer, ovarian cancer, melanoma and squamous cell carcinoma. D) Pilot studies are also being made in order to see if interferon effects can be potentiated by other biological modifiers.

All these patients are involved in various research programs taking place at different institutions.

PROGRESS: Interferon has an anti-tumor effect on humans. The studies now being conducted aim at studying how one should give the interferon therapy in an optimal way.

45. POLYVALENT PNEUMOCOCCAL VACCINE IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES OR DISORDERS OF IMMUNOGLOBULIN (ABBREV)

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Twenty patients with multiple myeloma (MM), 12 with chronic lymphocytic leukemia (CLL) and 21 normal controls received 0 cc of a 14 valent pneumococcal (pnc) vaccine (Pneumovax (registered trademark) Lot No. 0618A, and Merck, Sharp and Dohme). Pre- and post-vaccination type specific antibody (ab) levels were measured by RIA for 12 pnc types (Danish Nos. 1, 3, 4, 6A, 7F, 8, 9N, 12F, 14, 18C, 19F, 23F). Quantitative serum immunoglobulin (Ig) levels were determined for IgG, IgM and IgA by radial diffusion. Serial pharyngeal cultures were performed to document pnc carriage.

Except for types 3 and 12 in MM and type 3 in CLL, prevaccine pnc geometric mean ab levels were lower in patients than controls (p less than .05). The controls' post ab levels were significantly higher than CLL for all pnc types (p less than .05). Comparison of pre and post levels showed significant ab rises to all 12/12 pnc types in controls (p less than .05), to 1/12 (type 18) in MM and to 0/12 in CLL. There was no significant difference between early (1 mo.) and later (3-4 mo.) post levels indicating no delayed response. Serum Ig abnormalities were detected in 19/20 MM and 10/12 CLL patients. Chemotherapy was being received by 17/20 MM and 8/12 CLL patients. Serial pharyngeal cultures showed no difference in carriage rate between controls and patient groups.

MM and CLL patients show diminished ab response to pnc vaccine. This may be due to abnormal Ig production inherent to the disease and/or secondary to therapy.

46. TRANSFER FACTOR IN WALDENSTROM'S AND MULTIPLE MYELOMA

Silverman, M. A., University of Miami, School of Medicine, Dept. of Oncology, *1400 N.W. 10th Ave., Box 520875, Coral Gables, Florida*, 33152, United States of America

This is part of a broader project. A summary of this subproject is not available.

C. IMMUNOTHERAPY OF LUNG CANCER

47. IMMUNOSTIMULATION WITH BCG IN PRIMARY LUNG CANCER PATIENTS

Krzysko, R., SlowikGabryelska, A., Lukaszewicz, M., Gizycka, D., Regional Lung Diseases Control Centre, Immunological Department, *Szamarzewskiego 62, 60569 Poznan, Poland*

Intracutaneous BCG in different doses and time-intervals was given to two groups of primary lung cancer patients treated surgically and by radiotherapy/chemotherapy.

Remission rates and 3-year survival time with regard to BCG mode and histological type of tumor and treatment method are measured.

A control group without BCG in each subgroup is observed.

48. DIRECT INTRATUMORAL INJECTION OF CELL WALL SKELETON OF BCG OR NORCARDIA RUBRA IN LUNG CANCER IMMUNOTHERAPY

Hayata, Y., Taira, O., Hayakawa, K., Ogawa, I., Tokyo Medical College Hospital, Dept. of Surgery, *6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160, Tokyo, Japan*

In order to observe the regional efficacy of immunotherapy, cell wall skeleton of BCG (BCG-CWS) or Nocardia rubra (N-CWS) was injected intratumorally.

Two hundred micrograms of BCG-CWS or 500 micrograms of N-CWS was injected into the intrabronchial tumor using our special flexible needle at least two or three times under bronchofibroscopy. In peripheral tumors 500 to 1,000 micrograms of BCG-CWS or 1,000 micrograms of N-CWS was injected transthoracically once or twice a week for 2 to 4 weeks. One thousand to 3,000 micrograms of BCG-CWS or N-CWS was injected into the primary lesion or metastatic or invading lesions under thoracotomy.

In 61 bronchofibroscopically injected cases in which chemotherapy and/or irradiation was also employed, opening of the bronchial lumen and/or disappearance of the bronchial tumor was obtained at a much higher frequency than in cases in which chemotherapy and/or irradiation was administered without immunotherapy. Out of 12 peripheral tumors injected transthoracically, disappearance of tumor shadow was obtained in four.

Degeneration of intrabronchial tumor cells and decrease of intrabronchial tumors were significant in comparison to cases receiving live BCG or mitomycin C. We are continuing to observe the systemic efficacy of immunotherapy by intratumoral injection of BCG-CWS or N-CWS, and are compiling data on survival in comparison with our non-immunotherapy group.

49. IMMUNE STATUS IN LUNG CANCER - EFFECTS OF BCG IMMUNOTHERAPY AND COMBINED IMMUNOTHERAPY WITH BCG PLUS CHEMOTHERAPY (ABBREV)

Ricci, C., Mineo, T. C., Rea, S., Facciolo, F., Sacco, O., Policlinico Umberto I, Cattedra di Chirurgia Toracica, *Rome, Italy*

OBJECTIVES: Evaluation of the preoperative in vitro response to plant mitogens PHA and Con A of lymphocyte suspensions: effects and correlations with clinical response to BCG immunotherapy and immunochemotherapy (BCG plus BACON). Survival of patients with resected bronchogenic carcinoma modified by BCG immunotherapy and immunochemotherapy (BCG plus BACON).

APPROACH AND PROGRESS: Evaluation of the in vitro preoperative immunoresponse was done in 44 cases of lung cancer from July to December 1979 (28 were resected and 16 examined). We did not notice a significant difference in the in vitro immunoresponses of patients and control group. An inhibition of immunoresponse was found in the postoperative controls when patients were treated with immunochemotherapy and chemotherapy. The study has been too short and further investigations are necessary. Twenty-eight cases were admitted to our clinical trial (stage I: 8 cases; stage II: 3 cases; stage III: 17 cases): 1) Squamous cell carcinoma, stage I and II. The probability of survival in patients treated with resection and BCG immunotherapy has increased (70% versus 40% in control group). 2) Squamous cell carcinoma, stage III; anaplastic giant cell carcinoma, stage I, II, III; adenocarcinoma stage II and III. The immunotherapy alone has been found ineffective in these cases. In July we began a clinical trial with immunochemotherapy (BCG plus BACON), but the follow-up of these cases is still too short and further investigations are necessary.

50. IMMUNOTHERAPY OF PULMONARY TUMORS BY DIRECT INTRALESIONAL INJECTION OF BCG

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The purpose of this study will be to determine if the direct intralesional injection of BCG into lung tumors 2-3 weeks prior to pulmonary resection will enhance the tumor immunity and prolong survival. There is ample evidence to indicate that this is the case in animal experimental tumor systems and in other human neoplasms.

Fifty patients with pulmonary tumors have undergone direct intralesional injection of BCG into these tumors. Ten patients have been injected by way of the bronchoscope into endobronchial lesions and 40 patients have undergone direct percutaneous injection under fluoroscopic control. Patients undergo resection of the tumor 23 weeks following intratumor injection. Toxicity is closely related to the PPD reactivity. Patients who have a negative PPD have little or no toxicity. Those who have a positive PPD reaction experience temperature which persists from 24 hours to as long as 9 days. The degree of fever and the duration of fever are closely related to the magnitude of the PPD reaction. Therefore, the toxicity can be predicted by a PPD skin test. At the time of thoracotomy there was very little pleural reaction seen in the vast majority of patients. There was no postoperative empyemas or bronchial stump leaks and the postoperative convalescence was not prolonged.

We have begun to evaluate tumor infiltrating lymphocytes for surface markers and activity in MLC-CML assays, and data to date indicated some cytotoxicity and natural killer cell-like activity in lymphocytes infiltrating BCG injected tumors.

The total number of patients studied is 50. The total number of patients that were studied in the hospital is 50. The number of veterans is 18, and the number of non-veterans from UCLA is 32. No recombinant DNA is involved.

51. SURGERY, BCG AND PREDICTIVE TESTS IN LUNG CANCER

Bennett, J. A., Union University, Albany Medical College, School of Medicine, Depts. of Surgery/Physiology, *47 New Scotland Ave., Albany, New York*, 12208, United States of America

A system of predictive tests will be carried out in order to assess the response of patients to BCG and to analyze the biological activity in a particular batch of BCG. The patients to be studied will be lung cancer patients given BCG as adjuvant immunotherapy following surgical resection of their tumors. Several parameters will be tested and correlated with the clinical course of these patients. Host general immune competence will be analyzed by skin testing with phytohemagglutinin and enumeration of T lymphocytes. The potential of a patient to respond to BCG and the level of his response to BCG will be determined by the changes in the skin test response to PPD, in the blastogenic response of his lymphocytes to LPS, and in the number of T lymphocytes. The biological activity in a batch of BCG will be assessed by measuring its colony forming activity in vitro and by measuring in mice its inhibition of solid tumor growth, its antimetastatic potential, and its induction of suppressor cells.

The goals of this research are to develop, through laboratory tests, a data base that will establish guidelines for the pharmacological application of an immunopotentiator like BCG and to gain insights into the interactions of BCG with the host immune system.

52. PROTOCOL 34A - INTRAPLEURAL VS INTRADERMAL BCG IN PATIENTS WITH RESECTED NON-SMALL CELL CARCINOMA OF THE LUNG WITHOUT LYMPH-NODE METASTASES

Mendeloff, G. L., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *5000 W. National Ave., Wood, Wisconsin*, 53193, United States of America

Protocol 34A has been designed to evaluate the effect of non-specific immunotherapy on survival in male patients following curative resection for non-small cell carcinoma of the lung without lymph node metastases. Intrapleural or intradermal BCG will be utilized to challenge the immunological status of these patients. In addition, the time interval from resection to evidence of first recurrence will be analyzed. All male patients undergoing a curative resection (no microscopic evidence of residual disease) for non-small cell carcinoma of the lung without lymph node metastases (T1,2,3 N0M0) will be eligible for inclusion in this study. Prior to the 14th postoperative day, eligible patients will be randomized into one of three treatment groups: 1) A control group treated with only an operation, 2) a group treated with resection plus 10 to the 7th power BCG given intrapleurally followed by 300 mg INH every day for four months, 3) a group treated with resection plus the intradermal injection of 10 to the 8th power BCG every two weeks for 6 months. All groups will be followed in a similar fashion. Long-term survival will be utilized to measure the efficacy of the respective treatments. There is preliminary evidence to suggest that the intrapleural injection of BCG may prolong the disease free interval and ultimate survival in patients with 'curative' lung resections. Similarly other randomized studies would suggest that intradermal BCG may benefit patients in this same category. A logical extension of these facts would suggest comparison of the two adjuvant treatment modalities in a randomized study.

53. INTRATUMORAL BCG IMMUNOTHERAPY

Matthay, R. A., Yale University, School of Medicine, Dept. of Internal Medicine, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

OBJECTIVE: Evaluate in a controlled, randomized prospective study the therapeutic efficacy of intratumoral BCG prior to surgery in patients with carcinoma of the lung. The control patients will undergo conventional surgery for their tumors. At least 35 patients per year are expected to enter the study.

54. LEVAMISOLE AS ADJUVANT IMMUNOTHERAPY IN PATIENTS WITH MAMMARY CARCINOMA

TreurnietDonker, A. D., Amery, W. K., Blonk Van der Wijst, J., Bolhuis, R. L., Haije, W., Hop, W. C., Mak, A. C., Rotterdamsch Radiotherapeutisch Instituut, *Groene Hilledijk 301, Box 5201, 3075 EA Rotterdam, Netherlands*

Levamisole, a synthetic immunopotentiator, may have some value in the modulation of host immune resistance in the oncological patient.

This possibility is under investigation in a double-blind prospective randomized clinical trial on patients with mammary carcinoma.

A two-arm trial of levamisole versus placebo is applied to node-positive patients after surgery and radiotherapy for cure have been performed.

A battery of immunological tests is used regularly to monitor the host response during the two-year oral use of this adjuvant immunotherapy.

55. LEVAMISOLE INDUCED CHANGES IN IN VITRO/ IN VIVO IMMUNOLOGICAL RESPONSE IN PATIENTS WITH PRIMARY LUNG CANCER

Krzysko, R., SlowikGabryelska, A., Lukaszewicz, M., Regional Lung Diseases Control Centre, Immunological Department, *Szamarzewskiego 62, 60569 Poznan, Poland*

OBJECTIVE: In vivo/in vitro measurements of immunological parameters in patients with primary lung cancer undergoing classical treatment plus levamisole stimulation.

APPROACH: In each randomly selected patient at specified intervals skin tests (DNCB, PPD, candidine and streptokinase) (std) will be carried out, T-L populations, blastic transformation and migration inhibition will be observed and conclusions drawn as to whether levamisole has any stimulative effect on the above parameters.

PROGRESS: So far 110 patients have been examined. A correlation between the dosis and timing of levamisole and some of the parameters was observed. Levamisole seems to have a variable impact on immunological properties in lung cancer patients.

56. LEVAMISOLE AND LUNG CANCER

Cox, J. D., Kun, L., Greenberg, M., Gunn, W., Rangala, N., Wilson, J. F., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *5000 W. National Ave., Wood, Wisconsin*, 53193, United States of America

OBJECTIVES: To determine if adjuvant immunotherapy with levamisole will prolong the disease-free interval and improve survival following radiation therapy. To evaluate the anatomical sites of tumor recurrence following therapy to determine whether the addition of levamisole modifies the local tumor control with irradiation or the distant dissemination of bronchogenic carcinoma.

PLAN: To evaluate the short-term and late sequelae of radiation therapy alone or combined with levamisole.

METHODS: Patients with stages I-II will receive 6000 rads minimal T.D. in 6-7 weeks. Patients with stage III will

receive 5000 rads T.D. in five to six weeks. These groups will be randomized to receive an oral placebo or levamisole. Levamisole will be given in a dose of 2.5 mg/kg p.o.

FINDINGS: This protocol is still open. No conclusions or publications are available at present.

57. IMMUNOTHERAPY TRIAL OF LUNG CANCER PATIENTS WITH THE AID OF TRANSFER FACTOR AND LEVAMISOLE - A DOUBLE-BLIND STUDY

Krohn, K., Antila, P., Hakala, T., Raisanen, L., Uotila, A., Hakama, M., Tampere Yliopisto, Dept. of Biomedical Sciences, *Vuolteenkatu 11, 33100 Tampere 10, Finland*

OBJECTIVE: Immune therapy of lung cancer patients with the aid of transfer factor and levamisole.

APPROACH: One hundred sixty lung cancer patients will be treated for two years with transfer factor, levamisole and placebo preparations using a double blind method. The clinical status and the reactivity of cell mediated immunity are evaluated several times during the trial.

PROGRESS: About half the patients have been entered onto the study. The code will be opened after two years.

58. LEVAMISOLE, RADIATION AND INTRATUMORAL BCG THERAPY FOR ADVANCED LUNG CANCER

Papac, R. J., Matthay, R. A., Kapp, D. S., Fischer, J. J., Baue, A. E., Reynolds, H. Y., Raymond, L., Carter, D., Curtis, A., Loke, J., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Hematology/Oncology Service, *W. Spring St., West Haven, Connecticut,* 06516, United States of America

This project is a study to determine the effects of immunotherapy as an adjunct to radiotherapy in advanced bronchogenic carcinoma, non-anaplastic cell types. The forms of immunotherapy to be used are levamisole and levamisole with intratumoral BCG. Patients to be included in this study will be those with disease limited to the hemithorax with or without ipsilateral scalene node involvement and patients with stage I or II disease who are medically inoperable. The study will include a control group undergoing radiotherapy alone.

This study will involve patients in three institutions in this area, the Yale-New Haven Hospital, St. Raphael's and the Veterans Administration Hospital.

59. OAT CELL CARCINOMA - THYMOSIN IMMUNOTHERAPY

Sartiano, G. P., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Division of Hematology & Oncology, *Garner's Ferry, Columbia, South Carolina,* 29201, United States of America

This study is a chemotherapy-immunotherapy protocol in which patients with oat cell bronchogenic carcinoma are treated with aggressor chemotherapy (CAV and CCMV) in combination with radiation to primary and whole brain lesions (patients with limited disease) when indicated, and are randomized to additionally receive immunotherapy with thymosin (or a placebo).

Patients are staged following review of eligibility, and their categorization as limited or extensive type determined - both prior to study and again prior to radiotherapy, which is delayed until day 90 of treatment.

60. CONTROLLED THERAPEUTIC TRIAL IN PRIMARY LUNG CANCER - INOPERABLE TUMORS (PHASE III, PART 1)

Mirra, A. P., Miziara, J. E., Trippe, N., Schneider, C. A., Hospital A.C. Camargo, Depto. de Cirurgia Toracica, *Post Box 5171, R. Jose Getulio 211, Sao Paulo 01509, Sao Paulo, Brazil*

OBJECTIVES: To ascertain whether nonspecific immunotherapy in conjunction with chemotherapy and radiotherapy will improve the survival rate of patients suffering from advanced primary lung cancer.

APPROACH: The study includes all histologically confirmed cases of primary lung cancer among males of all ages at a specific clinical stage (UICC staging system). The patients undergo a series of examinations to assess the extent of the disease and their cellular immunology.

TYPES OF TREATMENT: Two types of therapy, (1) radiotherapy plus chemotherapy plus immunotherapy, and (2) radiotherapy plus chemotherapy. Chemotherapy is administered following the radiotherapy. Radiotherapy: (linear accelerator); 6,000 rad in two series of 3,000 rad each. Chemotherapy: Day 1 - Oncovin - 1.0 mg in 250 ml 5% glucose serum; Endoxan - 300 mg/m2/day - V.O. Day 2 - Adriblastina - 40 mg/m2 in 250 ml 5% glucose serum; Endoxan - 300 mg/m2/day - V.O. Days 3 and 4 -Endoxan -300 mg/m2/day - V.O. Day 5 - Oncovin - 1.0 mg in 250 ml 5% glucose serum; Enduxan - 300 mg/m2/day - V.O. Repeat this series every 21 days. Nonspecific immunotherapy: oral BCG. 500 mg/day for six days.

61. IMMUNOTHERAPY IN PATIENTS WITH NON-OAT CELL CARCINOMA OF THE LUNG (ABBREV)

Niell, H. B., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *1030 Jefferson Ave., Memphis, Tennessee,* 38104, United States of America

This protocol is to study the use of immunotherapy in patients with non-oat cell carcinoma of the lung. Early studies with some groups of European investigators have demonstrated that the use of immunotherapy after surgery in patients who have had resectable lesions for non-oat cell carcinoma of the lung has decreased recurrence rates in these patients. This has been achieved primarily with the use of levamisole, a nonspecific immunotherapy stimulant. The plan in this protocol is to randomize the patients with Stage I operable, Stage II inoperable, or Stage III inoperable non-oat cell carcinoma of the lung to either radiotherapy alone or radiotherapy plus C.parvum or radiotherapy plus levamisole. It is hoped that this protocol will then answer the question of whether immunotherapy has any place in the treatment of lung cancer.

62. A CLINICAL TRIAL IN APPARENTLY RESECTED PATIENTS COMPARING IMMUNOTHERAPY AND CHEMOTHERAPY AS ADJUVANT TO SURGERY

Oldham, R. K., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *1310 24th Ave. S., Nashville, Tennessee,* 37203, United States of America

OBJECTIVE: To determine whether the addition of radiation therapy and/or chemotherapy will be able to remove residual disease in a group of patients undergoing surgery for carcinoma of the lung in whom surgery cannot remove all evidence of tumor.

PLAN AND METHODOLOGY: Patients who have returned from surgery and in whom there is minimal disease remaining post-surgery (microscopic disease only) will be treated with either (1) radiation, (2) radiation plus Cytoxan (C), adriamycin (A), and platinum (P), or (3) Cytoxan, adriamycin and platinum alone. Previous studies have indicated that the combination of cytoxan and adriamycin is active in the treatment of metastatic non-oat cell lung cancer. Patients will be followed up after treatment for survival. Patients will receive six monthly courses of CAP.

CLINICAL RELATIONSHIP: This study is designed to determine whether the addition of radiation therapy or chemotherapy will improve the survival in patients undergoing lung resection in whom residual disease still remains.

63. ADJUVANT TUMOR SPECIFIC ACTIVE IMMUNOTHERAPY OF CARCINOMA OF THE LUNG

Takita, H., Bhayana, J., Gage, A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Surgical Service, *3495 Bailey Ave., Buffalo, New York,* 14215; United States of America

Following lung resection, patients with stage I and II disease are stratified by stage and histology and randomized into three groups: 1) control - conventional management; 2) specific immunotherapy -solubilized pooled allogeneic tumor-associated antigen with complete Freund's adjuvant; 3) non-specific immunotherapy - complete Freund's adjuvant only.

Since our report of 10/79 we have accrued 8 new cases of squamous cell carcinoma of the lung and 6 of non-squamous carcinoma. Among 70 squamous carcinoma patients currently evaluable, as of 10/1/80, there have been 13 failures (recurrence, metastasis or death) of 24 patients in group 1, 5 of 22 in group 2, and 6 of 24 in group 3. In a recent analysis of this data (8/80) the survival rates of groups 2 and 3 were significantly higher than those of group 1 (p less than .025), and there was a trend towards a longer disease free period (p equals .08).

Among 47 evaluable non-squamous carcinoma patients there have been 4 failures in group 1 (of 15 patients), 8 in group 2 (of 15 patients), and 3 in group 3 (of 17 patients). As stated in

our last report, we are no longer conducting a strictly randomized study in non-squamous carcinoma patients.

64. IMMUNE TESTING IN LUNG CANCER DURING IMMUNOTHERAPY

Harris, J. E., Braun, D. P., Plate, J. M., Rush University, School of Medicine, Dept. of Medicine, *1725 W. Harrison St., Chicago, Illinois*, 60612, United States of America

We propose to monitor, by immunological testing, surgically resected Stage I and Stage II lung cancer patients who will have been randomized into one of three 'treatment' arms. These arms are: (a) no further treatment, observation only; (b) three monthly immunizations with intracutaneous Freund's complete adjuvant; and (c) three monthly immunizations with intracutaneous Freund's complete adjuvant emulsified with tumor-specific or tumor-associated solubilized lung cancer cell membrane antigens. We will assess both general immune competence and specific antitumor immune competence. Tests of general immune competence will include (1) quantitation of the percent and absolute number of patient peripheral blood B cells, T cells and T cell subsets (TG and TM cells); (2) stimulation of lymphocyte proliferation in patient peripheral blood lymphocytes (PBL) by PHA mitogen and allogeneic leukocytes; (3) measurements of glass adherent, indomethacin-sensitive, 24-hour preculture-sensitive and ConA-inducible immunoregulatory suppressor cells in patient PBL; and (4) measurements of monocyte chemiluminescence in patient PBL. The tests for tumor-directed antitumor immune competence will include the leukocyte adherence inhibition assay (LAI), both the slide technique and the tube technique, and the leukocyte migration inhibition assay (LMI) utilizing both autologous and allogeneic lung tumor cell extracts. Each test will be applied in the post-surgical and post-vaccination period (a) to provide information about the mechanism of action of the immunotherapy used, (b) to clarify our understanding of the host tumor relationship, in particular to define those immunological mechanisms clearly related to the maintenance or augmentation of high levels of antitumor immunity, and (c) to assess the diagnostic and prognostic relevance of any or all of the testing procedures to be used. The results of our investigation will provide a clearer immunobiological appreciation of what may be involved in clinical trials of active specific immunotherapy in human cancer and indicate whether the approach does, in fact, result in any significant immunobiological changes which may be reflected in improved patient survival. (Text Abridged.)

65. OPSONIC AND PHAGOCYTIC ACTIVITY DURING TUMOR GROWTH

Saba, T. M., Weber, P. B., McKneally, M., Megirian, R., Blumenstock, F. A., Union University, Albany Medical College, School of Medicine, Dept. of Physiology, *47 New Scotland Ave., Albany, New York*, 12208, United States of America

The role of opsonic alpha 2SB glycoprotein as a factor regulating macrophage activity during experimental tumor growth was investigated. Parallel studies have also been done in patients with lung cancer to assess the serum level of opsonic alpha 2SB glycoprotein following BCG immunotherapy. The opsonic protein from mouse, rat and human serum has been isolated and an immunoassay for its measurement has been developed. Opsonic alpha 2SB glycoprotein is identical to cold-insoluble globulin or plasma fibronectin. Both proteins have identical molecular weights, manifest ability to stimulate particulate phagocytosis, and demonstrate heparin dependency in terms of biological activity. In vivo assessment of opsonin levels during tumor growth utilizing the Sarcoma 180 tumor model in mice demonstrated that with an increase in tumor growth there is an elevation in immunoreactive opsonic protein, but a decrease in the bioassayable activity of the plasma. Reticuloendothelial phagocytic activity was also measured during Sarcoma 180 tumor growth and activation of macrophage clearance was associated with increased hepatic Kupffer cell activity. Patients with lung cancer after lung resection but prior to BCG therapy demonstrated heightened levels of opsonic protein as measured by immunoassay. This heightened level post-operation was typical of rebound hyperopsonemia after surgery. With BCG, a normalization of opsonin levels was observed. Thus, macrophage activity may be, in part, modulated by circulating opsonin or plasma fibronectin but the association of cell bound fibronectin with phagocytic activity is yet to be determined. Also, during tumor growth there may be an inhibition of bioassayable opsonin activity even in the face of elevation of immunoreactive protein.

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66. IMMUNOTHERAPY OF SQUAMOUS CELL CARCINOMA

Takita, H., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

OBJECTIVE: Evaluate the therapeutic usefulness of solubilized lung cancer antigen and complete Freund's adjuvant in the treatment of Stage I and II lung cancer. This work will be done in the context of clinical protocols which have been approved by the Project Officer. Patients will be monitored for humoral and cellular immunologic changes during the course of their therapy.

67. IMMUNOTHERAPY OF LUNG CANCER

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This is part of a broader project. A summary of this subproject is not available.

D. IMMUNOTHERAPY OF BREAST CANCER

68. COMPARATIVE IMMUNOTHERAPY OF BCG VERSUS LEVAMISOLE IN BREAST CANCER

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OBJECTIVE: To determine the effectiveness of immunologic stimulation in breast cancer by measurement of disease-free interval, relapse rate and survival time. Also, to determine whether there are differences in non-specific immunotherapy with BCG or levamisole either in the patient's clinical course or immunologic reactivity.

APPROACH: Patients not previously treated are studied. All undergo surgery, and also radiotherapy and/or chemotherapy. They are divided among three treatment schedules: A) BCG (Institut Pasteur, Paris) 0.15 mg/dose, one dose every 14 days by scarification. B) Levamisole, 2.5 mg/kg body weight/day in cycles of two days of levamisole administration divided into three doses per day, followed by eight days of rest. C) Control.

All patients undergo treatment corresponding to the stage of disease as determined by the Hospital. Follow-up is expected to cover two years. Initially, 120 patients are expected to enter the study, which began in September 1979.

Patients will be stratified according to stage of disease, pre- or post-menopausal status, age, presence of estrogen receptors and histopathology of surgical specimens.

Erythrocyte sedimentation rate, complete hemogram, calcemia, serum alkaline phosphatose, complete urine and electrophoretic proteinogram will be determined routinely. Special studies performed every three months will include liver and skeletal scintigram, thoracic and skeletal teleradiography, determination of estrogen receptors and hypersensitive skin reactions to PPD and DNCB.

EVALUATION: Clinical evaluation will be made using disease-free interval, recurrence, and survival time, and occurrence of adverse reactions to medication, especially immune response indicators (for levamisole in particular, of the number of leukocytes; for BCG, control of localized reaction). Immunological reactivity will be measured by skin hypersensitivity to PPD 1/100 and DNCB (200, 100, 50 and 25 micrograms) after sensitization with a 1000 microgram dose, every three months. (Translated)

69. THERAPY OF CARCINOMA OF THE BREAST

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The objective of this proposal will be to determine if adjuvant immunotherapy with BCG alone or combination BCG immunotherapy and four drug chemotherapy will increase survival and prolong tumor-free interval in women with Stage II carcinoma of the breast, and to evaluate immunological parameters for following the clinical course and responses to this adjuvant therapy.

70. FOLLOW-UP TECHNIQUE FOR LEVAMISOLE IMMUNOTHERAPY OF POST-SURGICAL COLON AND BREAST CANCER, BY MEANS OF DNCB QUANTITATIVE TEST

de Carli, H. O., Restbergs, V., Hospital San Roque, Lab. Oncologia Experimental, *19 y 508 C.C. 350 Gonnet, 1900 La Plata, Argentina*

The main method for the immunotherapy follow-up is the quantitative DNCB test using, after sensitization, five paper disc with 200, 100, 50, 25 and 2.5 micrograms of DNCB. DNCB test and rosette E test were performed before and after levamisole administration. Including rest periods, treatment is continued until, if possible, the hyperergic state or positivity to 2.5 micrograms of DNCB is reached. A plotting of reaction grades vs. time is done.

In case lymph nodes are negative the hyperergic state is obtained within two months. When there are positive lymph nodes the hyperergic state is reached between the 12th and the 18th month. With metastasis the curve frequently shows a negative slope and the patient dies in spite of immunotherapy. Thus, the prognostic value of the shape of the curves has to be assessed, since a negative slope of the curves could signal a future metastasis.

The protection conferred by the hyperergic state to DNCB was proved in five (to eight) year survival rate causistics, using tumoral perchloric soluble glycoproteins and levamisole.

In minimum residual cancer, after the obtained normalization of immunological parameters, the posology of maintenance for indefinite time could be as follows: three 50 mg pills daily for three consecutive days and then a four day rest period; the regimen continued for three weeks; immunological control must be performed before and after every three weeks' treatment, i.e., yearly treatment consists of three weeks of drug administration and nine weeks of rest.

71. CANINE MODEL FOR HUMAN BREAST CANCER IMMUNOTHERAPY

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Studies of human breast cancer patients have shown that the biologic behavior of breast cancer reflects an interaction between the aggressive potential of the cancer cells and the tumor-retarding influence of host's tumor-specific cell-mediated immunity (CMI). More recent in vitro studies of leukocytes from human breast cancer patients indicate that simultaneous CMI to autologous breast cancer and the major envelope glycoprotein (gp55) of RIII-murine mammary tumor virus is prognostically beneficial, as judged by postoperative survival characteristics. These findings suggest that gp55 could be of value in immunotherapy and immunoprophylaxis of breast cancer. However, ethical considerations dictate that a test of the therapeutic potential of gp55 be made on an experimental model before clinical trials in humans. The natural behavior and structural characteristics of canine mammary cancer suggest that the dog provides an appropriate model. Preliminary in vitro studies have demonstrated that some breast cancer dogs have CMI to their autologous cancer and/or reactivity to selected human cancers and to gp55. Conversely, some breast cancer patients have CMI to canine mammary carcinomas.

The proposed study will seek to: (1) compare canine and human breast cancers in regard to structural, immunologic and biologic characteristics, and (2) determine whether therapeutic induction of gp55- and/or breast cancer-specific CMI in breast cancer dogs influences the biologic behavior of specifically characterized lesions.

72. CHARACTERIZATION OF HUMAN BREAST CANCER GLYCOPROTEINS

Bolognesi, D. P., Langlois, A. J., Collins, J. J., Roloson, G. J., Duke University, School of Medicine, Dept. of Surgery, *Box 3711, Durham, North Carolina*, 27710, United States of America

We have developed a unique model system for passive serum immunotherapy where immunological management of a highly lethal tumor, the ascites form of murine adenocarcinoma 755a, can be carried out reproducibly and with remarkable efficiency. Information concerning the mechanism of AD755a immunotherapy will be obtained through the isolation and characterization of the AD755a tumor-associated antigens and the serum factors responsible for providing protection. The type-C oncornavirus associated with the AD755a tumor will be isolated and the role of its structural polypeptides in the process will be determined.

The identity of AD755a tumor surface antigen(s) which induce and/or react with the protective serum will be analyzed by several analytical immunoassays, and attempts will be made to produce a protective serum using purified AD membrane antigens. The nature of the protective serum factor will be determined by isolating the class and subclass of antibody responsible for protection and the possible role of anti-idiotype antibodies in the serum will be analyzed.

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73. SOUTHWEST ONCOLOGY GROUP 7725 - CMPF CHEMOTHERAPY PLUS LEVAMISOLE FOR BREAST CANCER (ABBREV)

Coltman, C. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *San Antonio, Texas*, 78284, United States of America

This study is designed to determine the effect of levamisole on the duration of response and survival of patients with advanced breast cancer treated with maintenance chemotherapy. This study is designed to use the continuous Cooper regimen with Cytoxan, methotrexate, 5-FU, vincristine and prednisone as initial cytoreductive treatment for 8 cycles. After a response is observed, maintenance chemotherapy with oral 5-FU, methotrexate, cyclophosphamide, prednisone in 5 day cycles every 28 days will be instituted. A random half of those patients on maintenance phase of the study will then receive levamisole as an immune adjuvant. No patients have been entered into this study.

74. ADJUVANT RADIO-, CHEMO- AND IMMUNOTHERAPY OF OPERABLE BREAST CANCER

Grohn, P. T., Heinonen, E. O., Klefstrom, P. S., Tarkkanen, J. O., Helsinki University Central Hospital, Dept. of Radiotherapy, *Haartmaninkatu 4, Helsinki, Finland*

OBJECTIVE: The aim of this study is to evaluate the value of post-operative radiotherapy, chemotherapy, combined radio- and chemotherapy and all of the above with non-specific immunotherapy with levamisole.

APPROACH: Normally, operated on breast cancer patients with stages II of III receive postoperative regional radiotherapy. It is known that mostly in cases with state III and very often in cases with stage II breast cancer the spreading of the cancer has already taken place at the time of diagnosis for these patients the value of local radiotherapy is questionable, because radiotherapy affects local recurrences but not the life expectancy of the patients. The early results of adjuvant chemotherapy as obtained by Bonadonna and others are encouraging. In our study the patients are randomized to various treatment groups and levamisole is administered double-blindly.

PROGRESS: Stage II breast cancer patients are randomized to two treatment groups, both receiving postoperative radiotherapy with levamisole or placebo. The data are being evaluated . Stage III breast cancer patients are randomized to fol-

lowing treatment groups: postoperative radiotherapy or postoperative chemotherapy or both, together levamisole or placebo.

The patients are examined as to recurrent disease, immunological status, routine laboratory examinations.

The research with stage III breast cancer patients is in progress.

75. CHEMOIMMUNOTHERAPY OF ADVANCED BREAST CANCER

Oliveira Filho, J. A., Mineko, N., Mestre, M., Centro de Estudos e Pesquisas Oncologicas de Campinas, Dept. of Clinical Oncology, *Rua Alberto de Salvo, 311, Barao Geraldo, Campinas 13100, Sao Paulo, Brazil*

OBJECTIVES: Determine the efficacy in terms of response, duration of response, and survival of combination chemoimmunotherapy using cyclophosphamide, 5-fluorouracil, Adriamycin and BCG by scarification. Determine the quantitative and qualitative toxicity of the combination. Establish data on immunological status in these patients.

APPROACH: Patients with histologically confirmed carcinoma of breast, stage IV, with progressive disease without known CNS metastasis. Patients will be previously skin tested with DNB and a battery of recall antigens. The dosage schedule is as follows: 5-Fluorouracil - 600 mg/sqm IV day 1; Adriamycin - 50 mg/sqm IV day 1; cyclophosphamide - 600 mg/sqm IV day 1. Repeat cycle at 3 weeks' interval. BCG-C (Fundacao A. de Paiva - Rio Janeiro) 40 mg by scarification days 8 and 15 of each cycle. When the Adriamycin dose of 450 mg/sqm is reached it is discontinued. Methotrexate 50 mg/sqm day 1 and vincristine 1.5 mg/sqm day 1 are added and the therapy is continued for two years if there is no progression. This is a nonrandomized study. The protocol has been active since October 1978.

76. ADJUVANT CHEMOIMMUNOTHERAPY IN PRIMARY BREAST CANCER

Senn, H., Jungi, W. F., Amgwerd, R., Kantonsspital St. Gallen, Dept. of Medicine C, *CH9006 St. Gallen, Switzerland*

OBJECTIVES: To determine the influence of a 6-month chemotherapy program followed by BCG for 2 years on recurrence rates, tumor-free interval, survival and certain immune response parameters in primary breast cancer.

APPROACH: Two hundred and fifty-four women with T1-3a, N0-1 (positive or negative), M0 breast cancer were surgically treated by standardized modified radical mastectomy, stratified according to their axillary status into node-negative and -positive subgroups and then randomized to either receive no more treatment than surgical (controls) or receive 6 months of LMF (chlorambucil plus methotrexate plus fluorouracil) followed by monthly BCG-skin-scarifications until recurrence or for 2 years. All patients were examined at least every 3 months with a standardized follow-up program including a skin test battery.

RESULTS: At the last cutoff (June 30, 1978) after 2 1/2 years of median follow-up we observed: 1) A significant reduction in overall recurrence rate in node-negative cases in favor of the LMF/BCG treated subgroup (6.8% vs. 25.4% in surgical controls), 2) a trend, but no statistical difference, towards lower recurrence rates for the LMF/BCG subgroup in node-positive cases (32.2% vs. 45.6% in surgical controls), 3) virtually no reduction of recurrence rate by LMF/BCG in the high risk node-positive premenopausal patients (37.9% vs. 48.4% controls), 4) no difference in survival between adjuvant and control groups in both nodal subgroups at present.

The program continues with detailed follow-up analysis at 6 months.

77. CALGB PROTOCOL 7682 - COMBINATION CHEMOTHERAPY IMMUNOTHERAPY OF METASTATIC, RECURRENT, OR INOPERABLE CARCINOMA OF THE BREAST

Spaulding, M. B., Sanani, S., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *3495 Bailey Ave., Buffalo, New York,* 14215, United States of America

This study evaluates three different combinations for the treatment of metastatic or inoperable carcinoma of the breast (cytoxan, methotrexate, 5-FU with cytoxan, adriamycin, 5-FU, vincristine and prednisone) to determine whether remission frequencies were different, or durations of response were longer. One patient was entered at this institution. This is part of a larger study and there are no results available at this time.

78. THERAPY OF STAGE II AND STAGE III BREAST CARCINOMA

Scanlon, E. F., Evanston Hospital, *2650 Ridge Ave., Evanston, Illinois,* 60201, United States of America

GOAL: To determine the effects of adding adjuvant chemotherapy and BCG to mastectomy for patients with locally advanced breast cancer.

WORKSCOPE: After stratification for prognostic factors and menopausal status patients are assigned to one of three treatment schedules: (1) oral phenylalanine mustard (L-Pam); (2) 5-fluorouracil, cyclophosphamide and prednisone (CFP); and (3) CFP plus BCG inoculations. Two of these three treatment schedules are identical with the Mayo Clinic adjuvant study and the data will be cumulative. The treatment courses are given for one year or until there is evidence of recurrence, whichever occurs first. Survival and disease-free intervals will be computed for each treatment group.

79. NATIONAL SURGICAL ADJUVANT PROJECT FOR BREAST AND BOWEL CANCERS (NSABP)

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The National Surgical Adjuvant Project for Breast and Bowel Cancers (NSABP) is a multi-institutional (75 plus) cooperative cancer group with over 400 investigators conducting adjuvant breast and colorectal clinical trials. The group currently has 3 stage I and II breast cancer protocols open for entry which examine the merits of additive therapies such as chemotherapy, immunotherapy, and antiestrogens or more limited surgical procedures coupled with radiation therapy. The yearly accrual for 1977 will total about 950 patients, and follow-up now has reached 4,000 patients who were entered into earlier surgical and chemotherapeutic trials.

In addition, colorectal adjuvant studies are also being conducted utilizing multimodal treatments with 600-800 patients per year expected accrual. The protocols will compare groups receiving standard surgery (control arm) with immuno-, chemo- and radiotherapies. Patient accrual began in November 1977.

All these efforts are receiving the National Cancer Institute's support in terms of Headquarters and institutional grants and contracts. In addition, we have enjoyed support from the American Cancer Society which has enabled us to fund new members until more adequate long-term funds could be secured or to carry out important but discrete projects such as monitor estrogen-progesterone assay collection. The latter research is being supported by the American Cancer Society developmental grant (RD-14) and has greatly strengthened our tamoxifen (B-09) protocol by allowing us to scientifically monitor and perform quality assays on study patients.

In addition, the NSABP is about to analyze our first major surgical trial (B-04) at the five year point. We anticipate this report to be a major contribution to the surgical management of primary breast cancer. This would not have been possible without the America Cancer Society support we have received.

80. ADJUVANT CHEMOTHERAPY ANTIESTROGEN AND IMMUNOTHERAPY IN BREAST CANCER

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This is part of a broader project. A summary of this subproject is not available.

E. IMMUNOTHERAPY OF GENITOURINARY CANCERS

81. BCG IMMUNOTHERAPY OF RECURRENT SUPERFICIAL BLADDER CANCER

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Since January of 1978, 57 patients with transitional cell carcinoma of the bladder have enrolled in a randomized, prospective evaluation of intravesical and percutaneous BCG immunotherapy. Immunotherapy consisted of six 120 mg intravesi-

cal and 5 mg percutaneous Pasteur strain BCG administrations at weekly intervals as described by Morales et al. Side effects of BCG therapy are minimal, with approximately 91% mild dysuria, 83% urinary frequency, 39% mild gross hematuria, 22% low grade fever, and 17% nausea or vomiting. No disseminated BCG infection, allergic reaction, hepatic toxicity or cytopenia was observed and no patient required postponement of BCG treatments. Comparing the overall recurrence rate between groups, in the control group 46% had recurrent tumor, compared with 22% of the treated patients. Tumor recurrence was delayed with BCG therapy. Mean time to recurrence in the control is 12 months compared with 19.4 months after BCG (p equals 0.016, Wilcoxon). Immunological evaluation suggests that those who fail to develop cellular or humoral immunity to BCG related antigens have tumor recurrence at the same rate as controls: only 7% who converted PPD skin test and tumor recurrence compared with 44% of those who did not (p equals 0.056). Four of 5 BCG treatment failures had no in vitro immunity to PPD at the time of recurrence, and 2 of 5 had impaired humoral antibody response.

82. BCG IMMUNOTHERAPY OF RENAL CELL CARCINOMA

Lamm, D. L., Radwin, H. M., Banowsky, L. H., Shain, S. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Urology Section, *San Antonio, Texas,* 78284, United States of America

Renal cell carcinoma, which is responsible for approximately 7,000 deaths annually in the United States, is presently curable in less than 50% of patients. Currently available systemic treatments, both chemotherapy and endocrine therapy, result in response rates of 20% or less and complete and long-term responses are rare. The current study is designed to evaluate the potential role of Bacillus Calmette-Guerin (BCG) as an immunotherapeutic agent in the treatment of renal cell carcinoma. The present trial is justified in view of the demonstrated antigenicity of renal cell carcinoma, the well-documented instances of spontaneous regression, and the preliminary report by Eidinger and Morales, which indicates that 5 of 8 patients responded with 'objective evidence of improvement' following BCG immunotherapy of metastatic renal cell carcinoma.

Since the response to BCG immunotherapy is limited by the total number of tumor cells in many systems, an attempt to reduce the tumor burden is expected to improve the response to immunotherapy. In an effort to reduce the number of tumor cells, patients with metastatic disease will undergo surgical removal of the primary tumor and, in addition, patients will be given hormonal therapy with weekly medroxyprogestone acetate. As a corollary to this study, assays of androgen, estrogen and progesterone receptors will be performed on the excised tumor specimens.

83. IMMUNIZATION WITH BCG AND ALLOGENEIC RENAL CANCER CELLS

Oettgen, H. F., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

OBJECTIVES: Determine whether the cells of renal cancers have cross-reacting tumor-associated antigens by performing tests for humoral immunity to autologous tumor cells before and after immunization with BCG and allogeneic renal cancer cells. Attempt to analyze the specificity of the serological reactions and to investigate the patterns of antigen expression in terms of culture time, growth characteristics, and cell cycle kinetics. Periodic assessment of humoral immunity will be performed on sera from patients with Stage I renal cancer receiving conventional therapy and on sera from patients with Stage II, III, and IV renal cancer who are immunized with BCG and allogeneic renal cancer cells. It is expected that twenty patients per year will be entered on the study. Any modification of the laboratory or clinical studies must have the prior approval of the Project Officer before being instituted.

84. BCG IMMUNOTHERAPY - RECURRENT SUPERFICIAL BLADDER CANCER

Pinsky, C. M., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

OBJECTIVE: Evaluate in a controlled, randomized prospective study the therapeutic efficacy of intravesical and percutaneous BCG in patients with recurrent superficial bladder cancer. The administration of BCG shall be identical to the methods published in the J. of Urology 116: 180-183, 1976. It is expected that 32 patients per year will enter the study. Any modification of the protocol design must have the prior approval of the Project Officer before being instituted.

85. STUDY OF TRANSFER FACTOR THERAPY IN PATIENTS WITH CERVICAL CANCER

Gitsch, E., Wagner, G., Knapp, W., Tatra, G., Rainer, H., Universitat Wien, Dept. of Obstetrics & Gynecology, *Spitalgasse 23, 1090 Vienna, Austria*

OBJECTIVE: To investigate whether transfer factor therapy can improve the survival rate or delay the appearance of recurrent disease in patients with invasive squamous cell carcinoma of the cervix uteri.

APPROACH: Patients with histologically defined stage Ib or II of a squamous cell carcinoma of the cervix uteri receive about six weeks after radical operation (according to Latzko-Meigs or Wertheim) a radiotherapy by contact irradiation and/or teletherapy.

Immediately before radiotherapy and then every 4 weeks patients receive in a double-blind manner either placebo or 2E of a transfer factor (TF) preparation (1E is equal to TF from 10 to the 9th power leukocytes). TF is prepared from husbands of patients with cervical carcinoma who are immunologically tested and sensitized against DNCB and KLH before leukocyte donation.

Clinical parameters: delay in recurrence and increase in survival time. Immunological parameters: immune status before and after operation, after radiotherapy and then every three months; testing for transfered sensitivity against DNCB or KLH.

PROGRESS: So far 28 patients have entered the study. No side effects have been seen.

86. IMMUNOTHERAPY OF CANCER WITH IMMUNE RNA

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PROJECT OBJECTIVES: This research project was designed to see if there is a therapeutic benefit derived from the administration of xenogeneic immune RNA to patients at high risk of developing recurrence following curative resection for renal cell carcinoma. Patients admitted to this study had positive nodes at the time of nephrectomy, or have had capsullary invasion of the kidney by tumor.

RESEARCH PLANS: Xenogeneic immune RNA is extracted from sheep immunized with human renal cell carcinomas. Following nephrectomy, those patients are randomized to receive weekly intradermal RNA injections or to be included in a control group. Those receiving therapy receive treatment for one year. Survival and time to recurrence are being compared for each group.

METHODOLOGY: Patients receive skin testing to DNCB and common dermal antigens and have serum and lymphoid cells obtained and freeze-preserved at three-month intervals. Immune assays such as complement fixation to measure anti-renal cell cancer antibody and lymphocyte mediated cytolysis of renal cell cancers are being performed. An effort is being made to see if there are any changes in these donors which suggest protein synthesis or formation of antibody to a sheep idiotype. Immunization of sheep, RNA extraction, purification and storage of immune RNA, randomization, patient monitoring, and immunologic testing are all part of this study.

CLINICAL RELATIONSHIP: This adjuvant therapy study has a direct clinical relationship to the management of patients with renal cell malignancies. Adjuvant therapy is particularly attractive to this group of patients since virtually no effective systemic therapy exists for recurrent metastatic disease.

FINDINGS: There has been absolutely no demonstrable toxicity associated with treatment. Among the 12 patients who have received adjuvant immune RNA, there have been no recurrences. Follow-up will be required to see if this treatment is effective.

87. NON-SPECIFIC ADJUVANT IMMUNOTHERAPY IN THE RADIATION TREATMENT OF STAGE III CERVICAL SQUAMOUS CARCINOMA (ABBREV)

Shanta, V., Krishnamurthi, S., Girija, P., Nagarajan, B., Cancer Inst., Radiation Oncology Unit, *Adyar, Madras 600020, Madras, India*

Stage III cervical squamous cell carcinomas constitute 76% of all cervical cancers seen at the Institute. Radiotherapy per se has achieved so far only a 36% five-year disease-free survival in our series. This study was undertaken to evaluate whether a non-specific immunogenic agent like C. parvum would enhance cervical as well as pelvic tumor resolution.

Forty cases were entered into the trial, 20 study subjects and 20 controls. All were Stage III cervical squamous cell carcinomas. All received radiation therapy using a Cobalt-60 beam through 3 portals to deliver a mean tumor dose of 6,000 rads in the para-cervical cube, in about 6 weeks. No intracavitary therapy was used in any of these cases.

C. parvum was administered in the following protocol to the study group: 1) 20 mg I.V. infused in physiological saline one week prior to radiation; 2) 4 mg in physiological saline as I.V. bolus and 4 mg as intraparametrial injection weekly during the course of the radiation therapy; 3) 4 mg of C. parvum as intravenous bolus weekly for 8 weeks after the end of the radiation therapy.

The controls received only physiological saline on the same protocol as C. parvum in the study cases.

The criterion of favorable response was total clinical healing of the pelvic tumor within the volume of irradiation at 8 weeks after the end of the radiation therapy. The evaluation was blind.

A favorable response was obtained in 66.66% of the study cases and 62.5% of the controls. There was no therapeutic advantage in the C. parvum group. Apart from a pyrexial reaction on the day of the C. parvum injection no other toxicity was noticed.

88. CHEMOIMMUNOTHERAPY OF INOPERABLE CANCER OF THE VULVA

Silveira, J. G., Almeida, A. B., Silveira, G. G., Bolognesi, L. T., Pontificia Universidade Catolica do Rio Grande do Sul, School of Medicine, Dept. of Obstetrics & Gynecology, *Avda. Ipiranga 6681, Caixa Postal 1429, Porto Alegre 90000, Brazil*

OBJECTIVE: To improve the local conditions of the inoperable epidermoid carcinoma of the vulva, by reducing the ulceration and the edema and, thereby, to improve the urinary and rectal functions; to develop the use of immunotherapy as an adjuvant to chemotherapy.

APPROACH: All patients with proved epidermoid carcinoma of the vulva without conditions of operability are submitted to a four-drug five-day chemotherapy: Day 1: Bleomycin, 15 mg; Day 2: Vincristine, 1 mg/m2; Day 3: Cyclophosphamide, 400 mg, plus Methotrexate, 50mg; Day 4: Cyclophosphamide, 400 mg; and Day 5: Cyclophosphamide, 400 mg.

All drugs are injected intravenously and the treatment is repeated after a 3-week interval. If an objective response is obtained after three treatments (reduction of 50% of the ulceration or of the exophytic tumor), the protocol is continued for one year. All patients suitable for this protocol are randomized, every other patient receiving 150 mg of levamisole on days 2, 3, 4, 16, 17 and 18 of the interval.

89. IMMUNE COMPLEXES AND SPECIFIC ANTISERA IN OVARIAN CANCER PATIENTS - A PROGNOSTIC INDEX AND THERAPY MONITOR (ABBREV)

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OBJECTIVE: To evaluate whether immunoassay of circulating tumor-associated products in ovarian and other gynecological cancers can be used for diagnostic purposes in monitoring tumor burden and assessing responses to therapy.

APPROACH: Screening tests of C1q precipitation for immune complexes using serum and ascites fluid. Immunotherapy trial comparing two treatment groups: 1. Surgery plus chemotherapy. 2. Surgery plus chemotherapy plus BCG immunotherapy.

PROGRESS: No elevation of immune complexes has been found to be associated with ovarian cancer using direct C1q, indirect C1q, indirect heat inactivated or Raji assays. Levels have been found to be raised in other gynecological tumors, e.g., carcinoma of the cervix, vulva and body of the uterus. The BCG immunotherapy trial is in progress.

Fourteen patients have been treated on a double-blind basis with BCG or placebo. Results so far do not indicate any alteration of prognosis with intraperitoneal BCG.

90. POLYMERIZED TUMOR ANTIGEN-ADJUVANT IMMUNOTHERAPY

Neidhart, J. A., Wise, H., Ohio State University, Columbus Campus, School of Medicine, Dept. of Medicine, *370 W. 9th Ave., Columbus, Ohio,* 43212, United States of America

The presence of tumor-directed immune responses and absence of alternative modes of therapy make renal carcinoma an excellent model for immunotherapy in humans. Preliminary data from two institutions suggest that polymerized tumor antigens admixed with an adjuvant (PTA/Ad) can induce tumor regression and possibly increase survival in patients with disseminated renal carcinoma.

We propose a randomized prospective trial of PTA/Ad versus standard hormonal therapy in patients with measurable metastic lesions and a randomized double-blind trial of PTA/Ad versus adjuvant plus placebo in patients without clinical disease but with a high risk of recurrence. Frequency of treatments, dose of antigen, and the adjuvant will be changed to achieve evidence of general and tumor directed cellular immune reactivity as measured by serial skin tests and in vitro testing. Results of in vitro testing and skin tests will be correlated with disease status and clinical response by multivariate analysis. Statistical evaluation of clinical response, disease-free intervals, and duration of disease stability should allow determination of the efficacy of PTA/Ad immunotherapy in a human tumor model.

91. ADOPTIVE IMMUNOTHERAPY IN RENAL CELL CARCINOMA PATIENTS

Mannick, J. A., Richie, J. P., Steele, G., Wang, B. S., Zellen, M., Frei, E., Ervin, T., Wilson, R. E., Harvard University, Boston Campus, Peter Bent Brigham Hospital, Dept. of Surgery, *721 Huntington Ave., Boston, Massachusetts,* 02115, United States of America

No reliable means of therapy exists to date for patients with advanced renal cell carcinoma (RCC). This proposal concerns the application of the transfer of cellular immunity by immune RNA (I-RNA) in a randomized clinical trial in patients with renal cell carcinoma. Prior animal studies performed in our laboratory, utilizing a syngeneic mouse tumor host system, have indicated that immunotherapy with syngeneic spleen cells incubated in vitro with specific I-RNA raised in guinea pigs prevented death from pulmonary metastases in mice after excision of the primary tumor. In addition, Phase I studies of patients with metastatic RCC using the treatment plan detailed in this proposal have been completed. Preliminary results have demonstrated the following: 1) The I-RNA treated syngeneic lymphocyte infusions are well tolerated. 2) In vitro parameters of host anti-tumor response do reflect increased specific cell mediated cytotoxicity (CMC) against tissue-type specific tumor associated antigens after in vivo treatment. 3) There is no evidence of tumor growth acceleration after treatment. 4) Objective response of tumor regression has been observed in some patients after treatment. The proposed clinical study will be modeled on our experimental systems. Patients with Stage II RCC with extension into the inferior vena cava, Stage III RCC with positive regional lymph nodes, or Stage IV tumor with measurable disease in a prospective randomized trial will receive immune RNA therapy or standard therapy beginning approximately three weeks after excision of the primary tumor. I-RNA will be extracted from the lymphoid tissues of guinea pigs immunized against the patient's own tumor and will be incubated in vitro with the patient's peripheral blood lymphocytes. These autologus lymphocytes will then be returned to the patient intravenously after incubation. Correlation between in vitro parameters of tumor specific immunity and the patient's tumor-free survival will be sought utilizing tests for tumor specific CMC, complement dependent cytotoxicity, antibody dependent CMC and natural killer cell activity.

92. IMMUNOLOGIC INVESTIGATION OF HUMAN BLADDER AND KIDNEY CANCER

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The investigation of hybridoma anti-tumor antibodies as reagents for cytotoxicity and tumor localization now confirms that the anti-teratoma hybridoma antibody is capable of localizing teratocarcinoma in the murine host. Enzymatic modification of the antibody decreases the non-specific uptake by non-tumor organs and increases the excretion of tumor unbound antibodies. These developments indicate that the application for detection of tumors in humans may be warranted since the enzyme modified antibody would reduce irradiation to the host and the studies in vitro indicate that the murine anti-tumor antibody reacts against human teratocarcinoma.

Studies designed to estimate the risks to human tumor patients by use of these radio-labeled anti-tumor antibodies are under way in preparation for requesting permission for trials in patients with teratocarcinoma.

93. OMRF 7803 - ADVANCED METASTATIC RENAL CELL CARCINOMA (HYPERNEPHROMA) (HOD PLUS BCG TREATMENT REGIMEN)

Bottomley, R. H., Grozea, P. N., Hussein, K. K., Murray, J. L., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Hematology Section, *921 N.E. 13th St., Oklahoma City, Oklahoma*, 73104, United States of America

OBJECTIVES: To study the response of metastatic renal cell adenocarcinoma to combined hormonal therapy and chemotherapy with and without immunotherapy.

PLAN: Patients will be stratified according to performance status into two categories (grades 5-4 and grades 3-1), Treatment is with adriamycin every 3 weeks until a total dose of 450 mg/m2. Vincristine is given weekly for 5 treatments; then at 3-week intervals with adriamycin. Depo-Provera is given weekly. BCG is given every 2 weeks. Patients still in remission at the time of completion of their total dose of Adriamycin will receive maintenance with vincristine, BCG, and Depo-Provera. This maintenance regimen will be continued until relapse or complete response.

This study is still open. There are no V.A. patients on this study at this time.

94. IMMUNOTHERAPY IN OVARIAN CARCINOMA

Juillard, G. J., University of California, Los Angeles Campus, School of Medicine, Dept. of Microbiology & Immunology, *2025 Zonal Ave., Los Angeles, California*, 90024, United States of America

This is part of a broader project. A summary of this subproject is not available.

95. IMMUNOTHERAPY OF OVARIAN CARCINOMA

Knapp, R. C., Harvard University, Boston Campus, School of Medicine, Dept. of Radiation Therapy, *25 Shattuck St., Boston, Massachusetts*, 02115, United States of America

This is part of a broader project. A summary of this subproject is not available.

96. CHEMOTHERAPY WITH ADJUNCTIVE C. PARVUM AND AUTOLOGOUS VACCINE FOR HYPERNEPHROMA

Hemstreet, G. P., University of Alabama in Birmingham, School of Medicine, Dept. of Surgery, *1919 7th Ave. S., Birmingham, Alabama*, 35233, United States of America

This is part of a broader project. A summary of this subproject is not available.

F. IMMUNOTHERAPY OF GASTROINTESTINAL CANCERS

97. ADRIBLASTINA, 5-FLUOROURACIL AND ENDOXAN IN THE TREATMENT OF ADVANCED GASTRIC CANCER

Grandval, C. M., Hospital Nacional de Gastroenterology Bonorino Udaondo, Servicio de Cirugia, *Caseros 2061, Buenos Aires, Argentina*

OBJECTIVE: The high incidence of non-resectable stomach cancer (50 percent, or 112 of 223 operations) seen over the last three years in surgery at our hospital has prompted the use of numerous therapeutic protocols. Since they have not produced very satisfactory results in cases of advanced cancer, we have initiated a new program of chemotherapy.

METHODS: Patients with non-resectable stomach cancer undergo immediate post-operative polychemoimmunotherapy at dosages that are repeated every 5 weeks, with control of post-surgical development, results and toxicity. All patients received immunostimulants after chemotherapy (levamisole, 150 mg for 2 consecutive days each week). The proposed protocol is: 5-FU 12.5 mg/kg body weight i.m. daily for 5 days. Adriblastina 20 mg/m2 on day 1. Endoxan 400 mg/m2 i.v on day 5. This protocol is under way in 30 patients, with follow-up every 5 weeks. (Translated)

98. CELL MEDIATED INJURY TO COLON CANCER IN MAN - STUDIES OF MEDIATION AND ITS RELEVANCE TO IMMUNOTHERAPY

Doe, W. F., Dorsman, B., Temple, C., Royal North Shore Hospital of Sydney, Dept. of Medicine, *Pacific Hwy., St. Leonards, Sydney, N.S.W. 2065, Australia*

OBJECTIVE: To investigate the role played by inflammatory cells (IC), present within the tumor, in the control of growth and development of colonic carcinoma in man.

APPROACH: Specifically, 1) to recover, identify and quantify viable IC directly from colon cancer tissue and from macroscopically normal (control) colonic mucosa; 2) to show which of the IC types harvested from colonic tumors is capable of injuring colonic cancer cells in vitro; 3) to determine whether agents widely used for non-specific immunotherapy (BCG, Corynebacterium parvum, endotoxin) are capable of activating or amplifying the anti-tumor activity of individual IC types (especially macrophages) disaggregated from colonic carcinomas.

PROGRESS: Project has just commenced.

99. FOLLOW-UP TECHNIQUE FOR LEVAMISOLE IMMUNOTHERAPY OF POST-SURGICAL COLON AND BREAST CANCER, BY MEANS OF DNCB QUANTITATIVE TEST

de Carli, H. O., Restbergs, V., Hospital San Roque, Lab. Oncologia Experimental, *19 y 508 C.C. 350 Gonnet, 1900 La Plata, Argentina*

The summary for this project is under entry no. 70.

100. IN VITRO GENERATION OF IMMUNITY TO HUMAN COLON CANCER

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The projects encompassed by this application involve the characterization of the immune responsiveness to human colon cancer cells. The system will utilize 3M KCl solubilized antigens from a cultured colon adenocarcinoma cell, established and characterized by the applicant, encapsulated in neutral or charged liposome carriers. Normal peripheral blood mononuclear cells will be co-incubated with soluble antigens, liposome-encapsulated antigens, or antigen-fed macrophage preparations, and subsequently tested for specific antigen-induced blastogenesis or cytotoxicity on target colon tumor cells. The lymphoid cells acting in this in vitro system will be characterized for sheep cell rosettes (active and total T cells) and antibody-mediated killing (K cells). Isoelectric focused soluble tumor antigens will be tested in this system to discern inhibitory and/or stimulatory factors which could modulate the in vitro immunity. The isoelectric fractions which contain tumor-associated antigens, CEA, HLA, and fetal bovine sera and normal bowel antigens have already been identified with heteroantisera. A solid-state radioimmunoassay developed by the applicant will be utilized to characterize isolated histiotypic or individualistic tumor antigens. This approach to study of human tumor resistance, using a novel carrier, the liposome, may provide not only insight into the nature of immune induction by tumor antigens, but also clues to the biochemical and molecular identities of factors modulating immunity to surface antigens. An in vitro system for colon cancer may afford means to monitor tumor-associated antigens or their corresponding antibodies in afflicted patients, act as a screening procedure for purification of tumor-specific antigens, and provide reagents to monitor therapy protocols.

BIBLIOGRAPHIC REFERENCES: Kahan, B.D., Rutzky, L.P., LeGrue, S.J., and Tom, B.H.: New approaches to human colon cancer. Methods in Cancer Res. 18: 197, 1979.

101. REGIONAL K-FU AND HYPERTHERMIA FOR METASTIC COLON CANCER

Ramming, K. P., University of California, Los Angeles Campus, School of Medicine, Dept. of Surgery, *2025 Zonal Ave., Los Angeles, California,* 90024, United States of America

This research grant proposes innovative therapy for metastatic colorectal cancer, and also adjuvant immunotherapy for preoperative patients. Part A will compare the clinical response and palliation obtained in two groups of randomized patients with metastatic colorectal cancer confined to the liver. One group will be treated with conventional systemic 5-FU combined with hyperthermia, and the other will be treated with continuous infusion of 5-FU in the hepatic artery catheter combined with hyperthermia.

In Part B, colorectal malignancies are injected intralesionally with BCG or placebo prior to routine surgery. Survival and immune parameters are monitored postoperatively.

102. ADJUVANT THERAPY OF ADENOCARCINOMA OF THE COLON

Ramming, K. P., Stabile, B. E., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Surgical Service, *Wilshire & Sawtelle Blvds., Room 209, Los Angeles, California,* 90073, United States of America

This study was designed to carefully evaluate the role of adjuvant chemotherapy, chemoimmunotherapy, immunotherapy and radiotherapy in patients who are at high risk of developing recurrence following definitive surgery for colon cancer. Over 600 patients have been entered into this national study. Accrual to this study was stopped in August 1979 by unanimous national vote after assurances from our statistical center verified entry numbers to be sufficient for evaluations of survival, recurrence, and disease-free interval. The study remains active in that data collection and completion of therapy arms are still continuing. At present, differences in recurrence rates in two treatment arms remain somewhat higher but not significant. Staging via Dukes' classification has been proven to be of prognostic significance within this patient study group. There also appears to be a difference in recurrence rate by sex, with females having recurred more often than males. Survival and disease-free interval remain undetermined and additional data will need to be completed prior to any final analysis and unblinding of the coded data.

103. CLINICAL TRIAL OF POSTOPERATIVE IMMUNO- AND CHEMOTHERAPY FOR COLON CANCER (ABBREV)

Goldman, L. I., Golinger, R. C., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Surgical Service, *510 E. Stoner Ave., Shreveport, Louisiana,* 71130, United States of America

OBJECTIVE: This study will compare the length of the disease-free interval survival of patients who have had a curative resection of adenocarcinoma of the colon, situated entirely proximal to the peritoneal reflection, with that of patients who are receiving chemotherapy or immunotherapy in addition to the operation.

RESEARCH PLAN AND METHODOLOGY: The operations will be standard surgical procedures. Chemotherapy will consist of 5-fluorouracil, methyl CCNU, and vincristine, cycled at ten weeks times eight. Immunotherapy will consist of BCG scarification every week for the first three months, and then every other week thereafter. End points will consist of evidence of treatment failure, survival and morbidity. This is a part of a national surgical adjuvant protocol.

FINDINGS: To date, two eligible patients have been randomized. One accepted and has completed chemotherapy; the other has refused immunotherapy.

104. NSABP PROTOCOL C-01 - EVALUATION OF POST-OP IMMUNOTHERAPY AND SYSTEMIC CHEMOTHERAPY IN MANAGEMENT OF RESECTABLE COLON CANCER (ABBREV)

Cruz, A. B., Aust, J. B., Reed, R. C., Bannayan, G. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *San Antonio, Texas,* 78284, United States of America

For those patients receiving treatment, this study compares the length of the disease-free interval and survival of patients who have had a 'curative' resection of adenocarcinoma situated in the colon entirely proximal to the peritoneal reflection with that of patients who are receiving systemic chemotherapy or immunotherapy in addition to operation. Patients with Dukes' A and D colon lesions will be included in the study but only for information relative to pathology of their tumors.

To date, there have been eleven patients at Audie Murphy Veterans Administration Medical Center who have been entered into the NSABP colon protocol. Study is still ongoing and patient accrual is continuing. There are no early data available for disclosure at the present time. No particular problems, side effects, or untoward toxicity have been noted in the treatment regimen.

105. IMMUNOCHEMOTHERAPY OF COLORECTAL CARCINOMA (DUKES, ASTLER AND COLLER'S TYPES B2, C1 AND C2)

Periti, P., Universita degli Studi di Firenze, Istituto di Farmacologia e Tossicologia, *Viale Morgagni 65, 50134 Florence, Italy*

OBJECTIVE: The aim of this study is to evaluate immnochemotherapeutic treatment of Dukes, Astler and Coller's B2, C1 and C2 stage colorectal cancer after radical surgery. The study consists of a combination 5FU-BCG adjunctive therapy (Therapy 'A') which is compared to chemotherapy with successive cycles of four drugs (5FU, HYD, Me-CCNU, MTC), active in colorectal cancer, which are combined with BCG (Therapy 'B'). Toxicity of the two types of treatment used, together with their therapeutic response, expressed as disease-free interval, will also be studied. Post-surgical radiotherapy will be carried out before immunochemotherapy in the rectal cancer cases. It is expected that over a period of two years, 70 patients will be included in each type of treatment.

APPROACH: Introduction of cases into the study was begun on March 1, 1979. By November of the same year there were 42 patients under study, of whom 8 had rectal carcinoma and 34 colon cancer. Twenty-six patients were assigned to Therapy 'A' and sixteen to Therapy 'B.' The patient group includes 24 females and 18 males, average age 58 years. Eighteen patients have been under study for 3 months, nine for 6 months and one patient for 9 months. Light or moderate nausea, vomiting and blood toxicity were seen in some cases during immunochemotherapy. Two patients undergoing radiotherapy showed signs of toxicity (ray cystitis in one case and ray sickness in the other).

106. THE EFFECT OF SURGERY COMBINED WITH RADIOTHERAPY, CHEMOTHERAPY AND MER ON PATIENTS WITH COLON AND RECTOSIGMOID CANCER (ABBREV)

Robinson, E., Rambam University Hospital, Dept. of Oncology, *Haifa, Israel*

OBJECTIVE: To increase the disease-free interval and the cure rate of patients with colon and rectum cancer with high likelihood of recurrence.

APPROACH: Patients with Dukes B2 and C after operation are randomized into 2 groups, one receiving radio-, chemo- and immunotherapy and the second receiving only chemotherapy. Immune investigation includes skin test and lymphocyte transformation studies; E rosette tests will be performed on all the patients before and after therapy. Survival, disease-free interval and immune studies of the patients in both groups will be evaluated.

107. COLORECTAL CANCER TRANSFER FACTOR FEASIBILITY STUDY

Yonemoto, R. H., Chee, D. O., City of Hope Medical Center, Dept. of Clinical Immunology, *1500 E. Duarte Rd., Duarte, California,* 91010, United States of America

The applicability of transfer factor (TF) for colorectal carcinoma will be explored by an in-depth study rather than by a large scale clinical trial. A horizontal study will be performed

on two groups of patients: 1) Post-op less than 3 years free of disease and 2) recurrent disease. The incidence and degree of 'serum blocking factors' (SBF) according to stage, disease-free interval and tumor burden will be assessed utilizing the leukocyte adherence inhibition (LAI)-blocking assay. By random selection, TF from three sources - 1) 'cured' colorectal carcinoma, 2) 'cured' breast carcinoma and 3) normal control - will be administered to both groups of patients twice, 7 days apart, following which the effects of TF on LAI and SBF will be assessed. The unblocking effect of TF will be measured by an in vivo and in vitro assay technique. These studies could explain the mode of action of TF (? unblocking) and the specificity of TF.

A vertical study will consist of investigation of patients undergoing curative resection of colorectal carcinoma. Pre- and postoperative serial LAI-blocking assays will be correlated with stage of disease, tumor burden and CEA.

LAI-blocking assay may serve as a prognosticator and an adjunct to treatment of colorectal carcinoma.

108. CHEMOIMMUNOTHERAPY WITH 5-FU/MMC/ CCNU/LEVAMISOLE FOR ADVANCED GASTROINTESTINAL CANCER

Oliveira Filho, J. A., Mestre, M., Modesto, N., Centro de Estudos e Pesquisas Oncologicas de Campinas, Depto. de Oncologia Clinica, *Rua Alberto de Salvo, 311, Barao Geraldo, Campinas 13100, Sao Paulo, Brazil*

OBJECTIVE: Determine the efficacy of the combination 5-fluorouracil, mitomycin-C, CCNU and levamisole (5-FU/ MMC/CCNU/LEVAMISOLE) for advanced gastrointestinal cancer. End points: Response/remission rate, toxicity/morbidity of treatment, survival rate, duration of survival, objectve tumor response.

PROTOCOL OUTLINE: Non-randomized study. Arm 1: 5-FU/MMC/CCNU/LEV. Dosage schedule: 5-FU 500 mg/sqm IV on day 1 and q. 15 ds. MMC 30 mg/sqm IV on day 1 and q. 15 days. CCNU 130 mg/sqm p.o. on day 1 and q. 45 days. LEV 100 mg/sqm p.o. on days 7-8-9 and q. 15 days. Protocol entry criteria: Measurable, progressive, metastatic, non-resectable gastrointestinal carcinoma.

CURRENT STATUS: Active since June 1978 (a total of 100 patients will be entered).

109. ADJUVANT CHEMOIMMUNOTHERAPY OF LARGE BOWEL CANCER

Koyama, Y., Hojo, K., Moriya, Y., Pitelka, F. A., Ministry of Health & Welfare, National Cancer Center, *5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Tokyo, Japan*

OBJECTIVE: Improvement of the end results of colorectal cancer treatment.

APPROACH: Pre- and postoperative long-term administration of mitomycin C, 5-FU and/or its derivatives. Evaluation of additional immunotherapy using BCG, picibanil (cell body component of Streptococcus).

PROGRESS: Control study has been conducted since 1975. Number of patients entered into the study - about 150 cases.

110. NATIONAL SURGICAL ADJUVANT PROJECT FOR BREAST AND BOWEL CANCERS (NSABP)

Fisher, B., University of Pittsburgh, School of Medicine, Dept. of Surgery, *3550 Terrace St., Pittsburgh, Pennsylvania,* 15261, United States of America

The summary for this project is under entry no. 79.

111. HEPATIC DEARTERIALIZATION, INTRAPERITONEAL BCG AND CHEMOTHERAPY FOR THE TREATMENT OF EXTENSIVE INTRA-ABDOMINAL CANCER WITH LIVER METASTASES

Neshat, A. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Subsection, *Asheville, North Carolina,* 28805, United States of America

Since the start of this study, 10 patients have been treated. All patients had intra-abdominal cancer due either to GI tract, biliary tract, or pancreatic tumors. All other cancers were excluded. All patients had liver metastases. The hepatic artery was ligated above the take-off of the gastroduodenal artery and cholecystectomy was performed to prevent ischemic necrosis of the gallbladder. At the time of closure of the abdominal cavity, a small French catheter was left inside the abdomen over the dome of the liver, and one ampul of Glaxo BDG diluted in 20 cc water was instilled through the French catheter which was then removed. Beginning five to seven days post-operatively the patients received 5-FU intravenously for five days. The patients tolerated this procedure quite well. Liver enzymes were elevated a few days after surgery and all patients received antibiotics. No serious complications and no deaths resulted from this procedure.

All ten patients have died. The longest survival was 23 months and the shortest two months. New patients are still being treated in the study.

G. IMMUNOTHERAPY OF HEAD AND NECK CANCERS

112. TREATING HEAD AND NECK EPIDERMOID CANCERS WITH METHOTREXATE, INH, BCG

Suen, J. Y., Westbrook, K. C., Wetmore, S. J., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *300 E. Roosevelt Rd., Little Rock, Arkansas,* 72206, United States of America

We are participating in VA Protocol 31, which is a cooperative program comparing methotrexate to methotrexate plus MER. During the calendar year 1979, 4 patients were started in this protocol. A total of 15 patients have been entered into this study and 11 of them have died. Of the 4 that are currently alive, 2 are receiving terminal care, 1 has a second primary tumor, and the last patient is without apparent tumor at the date of this report. As of December 21, 1979, Protocol 31 has been officially discontinued.

113. EFFECTS OF RADIATION THERAPY ON CELLULAR AND HUMORAL IMMUNITY OF PATIENTS WITH HEAD AND NECK CANCER (ABBREV)

Eells, R., McLaren, J., Olkowski, Z., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Radiology Service, *1670 Clairmont Rd., Decatur, Georgia,* 30033, United States of America

Accession of new patients has been closed, with 62 evaluable cases. The group randomized to adjuvant BCG/levamisole and the group receiving radiotherapy alone were reasonably comparable as to age, sex, site and stage. At entry, 64% showed impaired immunocompetence (as compared to age-matched controls without cancer). To date, there have been no significant differences between the immunotherapy and radiation only groups with respect to tumor control, disease-free interval, or survival curves. There were some variations between the two groups in the immunologic perameters, but these were not statistically significant. In vitro test results for all patients were as follows: T cell absolute numbers decreased at entry, with further fall at end of radiation, but recovery occurring at 8 weeks; T cell percentages decreased at entry, with no further changes after radiation or during follow-up to 40 weeks; B cell absolute numbers decreased at end of radiation, but with subsequent recovery maintained through 40 weeks; B cell percentages showed no significant alterations; blastogenesis (with PHA and PWM) decreased at entry, with further fall after radiation, and partial recovery during follow-up through 28 weeks; sialic acid levels elevated, with slight further rise at end of the radiation and gradual decrease toward normal at later follow-up. Lymphocyte cytotoxicity showed no apparent radiation effects, but has been discontinued because of complexity and variability of the results. Median survival had not been reached at last review. Monitoring of patients continues with periodic immunologic profiles. Futher correlation of test results with clinical course and outcome with be made.

114. IMMUNOCHEMOTHERAPY WITH MTX AND CORYNEBACTERIUM PARVUM FOR HEAD AND NECK CARCINOMA POST-SURGERY AND - RADIOTHERAPY

Oliveira Filho, J. A., Mestre, M., Centro de Estudos e Pesquisas Oncologicas de Campinas, Depto. de Oncologia Clinica, *Rua Alberto de Salvo, 311, Barao Geraldo, Campinas 13100, Sao Paulo, Brazil*

OBJECTIVE: Determine both the toxicity and the degree of antitumoral activity of immunochemotherapy with MTX and

C. parvum for head and neck carcinoma post-surgery and radiotherapy.

PROTOCOL OUTLINE: Non-randomized study.

APPROACH: Arm I: MTX plus C. parvum.

DOSAGE SCHEDULE: Methotrexate 40 mg/m2 I.V. Days 1 and 15 or 3 mg/m2 P.O. days 1-5. C. parvum 4 mg/m2 I.M. days 7 and 21. Dosage modification for toxicity will be given.

SPECIAL STUDY PARAMETERS: Skin tests.

END POINTS: Complete and partial response rates, duration of response, objective tumor response, toxicity/morbidity of treatment, survival time, survival rate.

CURRENT STATUS: Active since October 1978.

115. ADJUVANT IMMUNOTHERAPY IN THE TREATMENT OF PATIENTS WITH HIGH RISK MALIGNANT MELANOMA OF THE HEAD AND NECK, AND OF THE EXTREMITIES

Papac, R. J., Ariyan, S., Mitchell, M. S., Stansel, H. C., Glickman, M., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Hematology/Oncology Service, *W. Spring St., West Haven, Connecticut,* 06516, United States of America

The aim of this study is to determine whether immunocompetence of melanoma patients at the time of diagnosis is of predictive prognostic importance, and whether immunostimulation with direct stimulation of draining nodes as well as by adjuvant immunotherapy will improve survival or disease free interval in malignant melanoma. The proposed study will include patients with head and neck melanomas, allocated on a random basis to receive monthly MER, intraoperative MER or both in addition to surgical intervention in a control surgical group. Cases of extremity melanomas also will be studied on a random basis to receive excision with perfusion of DTIC, and MER as well monthly, or intraoperative MER or both. The nodular and the spreading forms of melanomas will be segregated in the study.

Immunologic testing is to include in vitro testing of patients' lymphocytes against melanoma cells in culture, as well as skin testing.

116. IMMUNO-STIMULANTS IN LOCALIZED RADIATION THERAPY

Wara, W. M., University of California, San Francisco Campus, School of Medicine, Dept. of Radiation Oncology, *551 Parnassus Ave., San Francisco, California,* 94122, United States of America

GOAL: To document the immunosuppression which occurs following radiation therapy to determine if a therapeutic advantage can be achieved by the addition of immunotherapy (thymosin) to the conventional treatment given for squamous cell carcinoma of the head and neck region and esophagus.

APPROACH: Both groups of head and neck and esophageal cancer patients will receive radiation therapy and/or surgery as treatment. Patients will then be randomized to receive thymosin or placebo in addition to standard treatment. All patients will be monitored with immunologic tests to measure antibody-mediated and cell-mediated immunity at regular intervals. The data will be analyzed for local disease recurrence, metastatic disease, and survival difference in the treatment groups.

117. LEVAM AS THERAPEUTIC ADJUNCT IN SQUAMOUS CARCINOMA

Strong, E. W., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

The overall goal of this study is to improve the recurrence and survival rates of patients with epidermoid cancer of the head and neck by use of an immunopotentiator. A second major goal is to investigate immune reactivity in these patients and to correlate this with stage and site of disease, and with the clinical progress of the disease.

118. INTRATUMORAL IMMUNOTHERAPY PRIOR TO SURGERY

Neifeld, J. P., Lawrence, W., Blievernicht, S., Kaplan, A. M., Virginia Commonwealth University, Medical College of Virginia, Dept. of Surgery, *1200 E. Broad St., Richmond, Virginia,* 23298, United States of America

This protocol should determine whether or not presurgical intralesional treatment with C. parvum in conjunction with post-surgical C. parvum as an adjuvant to surgery in the treatment of epidermoid carcinoma of the head and neck will 1) decrease the incidence of a) local recurrence and b) distant metastases, 2) prolong the disease-free interval, and 3) increase the overall cure rate of the tumor. The experiments carried out in conjunction with this protocol will provide information on the prognostic value of monocyte chemotaxis in epidermoid carcinoma of the oral cavity, pharynx, and larynx. Moreover, these experiments should help elucidate some of the effects of C. parvum on monocyte function and antitumor effector function in man.

BIBLIOGRAPHIC REFERENCES: Pre-surgical Intratumor Immunotherapy of Carcinoma of the Head and Neck. G. Schuller, C. Squires, C.H. Moy, P. Brown, J. David Beatty, J.J. Terz, and A. Kaplan. Medical College of Virginia. Proc. Am. Assoc. of Cancer Research 19:223, 1978. Adjuvant Intralesional and Systemic Corynebacterium Parvum Immunotherapy for Surgically Treated Head and Neck Cancer. J. David Beatty, J.J. Terz, P. Brown, W. Lawrence, G. Schuller, and A. Kaplan. Surgical Forum 29:155-157, 1978.

119. PHASE I COMBINATION CHEMOTHERAPY WITH IMMUNOTHERAPY IN HEAD AND NECK CANCER (HUMAN)

Zighelboim, J., University of California, Los Angeles Campus, School of Medicine, Dept. of Medicine, *2025 Zonal Ave., Los Angeles, California,* 90024, United States of America

This is part of a broader project. A summary of this subproject is not available.

H. IMMUNOTHERAPY OF OTHER AND MULTIPLE CANCERS

120. IMMUNOLOGICAL STUDIES ON CANCERS FREQUENTLY OCCURRING IN INDIA - ORAL CANCERS

Gangal, S. G., Nair, P. N., Rao, R. S., Cancer Research Inst., Biology Division, *Dr. Ernest Borges Marg, c/o Tata Memorial Ctr., Bombay 400012, Maharashtra, India*

OBJECTIVE: In our earlier studies we reported that oral cancer patients show cellular sensitization to autochthonous tumor extracts. The antitumor response can be maintained for 3-4 weeks in patients treated with surgery by intradermal inoculation of small doses of antigen. Experiments to increase antitumor immunity further, by inoculating patients with cauterized tumor antigen and BCG, have been undertaken.

APPROACH AND PROGRESS: Ten oral cancer patients treated with surgery received 5 mg of tumor antigen and 1 mg of BCG 2 days after operation and every 3 months thereafter. Control group was inoculated with BCG alone. Patients followed up for a year showed presence of tumor associated reactivity only in the group inoculated with BCG and tumor antigen. Marginal and temporary serum blocking activity could be demonstrated. At the end of one year, 3 patients from the experimental group remained tumor free and showed elevated antitumor immunity.

121. CLINICAL TRIAL OF RADIATION THERAPY AND CORYNEBACTERIUM PARVUM

Suit, H. D., Wang, C. C., Raker, J., Massachusetts General Hospital, Dept. of Radiation Medicine, *32 Fruit St., Boston, Massachusetts,* 02114, United States of America

Analysis of the disease free survival and local control frequency of 59 patients entered into the prospective and randomized trial of intravenous and intralymph node C. parvum combined with radiation therapy or radiation therapy alone for squamous cell carcinoma of the pharynx and sinus has shown no advantage for the C. parvum groups. This is true for all stages of disease and anatomic sites. In fact, there is a tilt in favor of the radiation alone group for the less advanced disease patients. Patients who experienced a temperature in excess of 104 degrees F did less well than patients whose temperature remained at lower levels. A group of 15 patients with locally advanced squamous cell carcinoma of the oral cavity or pharynx were treated by an intensive schedule of C. parvum; 15 intravenous injections of 4mg/m2 at 3 injections per week. Radiation was given 3 times per week and was administered at the peak of

febrile response. These patients have not done better than comparable patients treated previously by radiation alone. Laboratory studies are progressing on 9 spontaneous tumors of C3Hf/Sed mice. TD50 studies are complete on two, and they demonstrate a weak immunogenicity. C. parvum administration after transplantation has achieved only slight delays in several of these tumor systems.

122. TRANSFER FACTOR FROM INDIVIDUALS RECOVERED FROM INFECTIOUS MONONUCLEOSIS AS AN ADJUVANT IN RADIOTHERAPY OF NASOPHARYNGEAL CARCINOMA

Ho, J. H., Goldenberg, G., Chau, J. C., Kwan, H. C., Queen Elizabeth Hospital, *Kowloon, Hong Kong*

OBJECTIVE: To test the effectiveness of the transfer factor as an adjuvant in the radiotherapy of NPC patients with cranial nerve or base of skull involvement with or without cervical nodal involvement above the supraclavicular fossae. The transfer factor and placebo are prepared by the Manitoba Institute of Cell Biology and shipped to the Institute of Radiology without label so that the trial will be double-blind.

123. SURGERY, REGIONAL BCG, AND SYSTEMIC VACCINE THERAPY

McKneally, M. F., McIlduff, J. B., Kausel, H. W., Lininger, L., Bennett, J. A., Alley, R. D., Older, T. M., Foster, E. D., Hussain, M., Union University, Albany Medical College, School of Medicine, Dept. of Surgery, *47 New Scotland Ave., Albany, New York,* 12208, United States of America

The effect of immunostimulation will be tested as an adjunct to surgical resection will be tested in thoracic surgical patients with no evident disease after surgical removal of their tumors. As an extension of our prior studies with intrapleural BCG immunostimulation, patients with resectable cancers of the lung and esophagus will be treated with intrapleural BCG as regional nonspecific immunotherapy, followed by two vaccinations of autologous tumor cells. These cells will be reproductively terminated by x-irradiation, but will be viable, and will be administered in combination with BCG in an optimal ratio to elicit specific immunization. By this means we seek to protect from disseminated micrometastases. Patients will be randomly assigned to receive immune therapy after resection, or to enter the control group treated by surgery alone. They will be followed by clinical criteria and skin tests at regular intervals to determine time to recurrence or death. This clinical program will be carried out in collaboration with the NCI Frederick Cancer Research Center. Mutual exchange of visits by the scientific staff of both institutions is planned to facilitate the continuing development and application of a relevant animal model of human cancer, and to catalyze the rapid translation of laboratory advances to the clinical setting.

124. A CONTROLLED CLINICAL TRIAL OF IMMUNOTHERAPY IN RESECTABLE BRONCHIAL CARCINOMA

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OBJECTIVE: a) To measure the effect of AIT on cancer directed and non-specific tests of immunological function. b) To determine whether AIT delays local recurrence and metastatic spread or prolongs survival. c) To determine whether there is any correlation between the effect of this treatment on clinical progress and the effect on immunological function.

APPROACH: Patients undergoing surgical resection for bronchial carcinoma are randomly allocated to treatment and control groups. Both groups have serial measurements of specific and non-specific cell-mediated immunity and regular assessment of clinical and radiological evidence of local recurrence and metastatic spread. Administration of autograft: BCG and cells are injected into alternate extremities in turn as in the MRC Leukaemia Trials protocol.

PROGRESS: To date insufficient cases exist for meaningful survival curves to be drawn. Administration of autograft does not appear to have any long-term side effects. Apart from determining the role of AIT cells in bronchial carcinoma, this study is yielding useful results in determining the sensitivity and specificity of current tests of immunological status.

125. CHEMOIMMUNOTHERAPY OF BRONCHOGENIC CARCINOMA WITH CCNU (LOMUSTINE), PROCARBAZINE AND C. PARVUM

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OBJECTIVE: Evaluate the efficacy of the combination of chemo- and immunotherapy in prolongation of the disease-free interval and survival of advanced bronchogenic carcinoma patients.

APPROACH: Patients with histologically confirmed epidermoid carcinoma of the lung in an advanced stage will enter this protocol. This combination was chosen because of the high rate of CNS metastasis in lung cancer, the associated immunodepression in advanced disease and the known action of the two cytotoxic agents and stimulation properties of C. parvum. Patients will be previously skin tested with DNCB and a battery of recall antigens, repeated at 3 month intervals. The dosage schedule is as follows: CCNU 100 mg/sq.m p.o. day 1, max. 200 mg every 6 weeks. Procarbazine 100 mg/sq.m p.o. day 1 through 14 beginning with 50 mg, escalating 50 mg a day - max. 150 mg. C. parvum 2 mg/sq.m i.m. days 7, 21 and 35. Repeat cycle at 6 week intervals. This protocol has been active since October 1978.

126. EFFECTS OF RADIATION THERAPY ON CELLULAR AND HUMORAL IMMUNITY OF PATIENTS WITH BRONCHOGENIC CARCINOMA (ABBREV)

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Accession of new patients has been closed, with 61 evaluable cases. The group randomized to adjuvant BCG/levamisole and the group receiving radiotherapy alone were reasonably comparable as to age, sex, site and stage. At entry, 82% showed impaired immunocompetence (as compared to age-matched controls without cancer). To date, there have been no significant differences between the immunotherapy and radiation only groups with respect to tumor control, disease-free interval, or survival curves. There were some variations between the two groups in the immunologic perameters, but these were not statistically significant In vitro test results for all patients were as follows: T cell absolute numbers decreased at entry, with further fall at end of radiation, but recovery occurring at 8 weeks. T cell percentages decreased at entry, with no further changes after radiation or during follow-up to 40 weeks; B cell absolute numbers decreased at end of radiation, but with subsequent recovery maintained through 40 weeks; B cell percentages showed no significant alterations; blastogenesis (with PHA and PWM) decreased at entry, with further fall after radiation, and partial recovery during follow-up through 28 weeks; sialic acid levels elevated, without further change at end of the radiation or on later follow-up. Lymphocyte cytotoxicity showed no apparent radiation effects, but has been discontinued because of complexity and variability of the results. Median survival had not been reached at last review. Monitoring of patients continues with periodic immunologic profiles. Futher correlation of test results with clinical course and outcome will be made.

127. BRONCHOGENIC CARCINOMA - IMMUNOLOGIC PROFILE, RADIOTHERAPY AND IMMUNOTHERAPY

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This study compares the effect of the BCG immunotherapy given in conjunction with radiotherapy (BCG-RT) with that of radiotherapy (RT) alone. Patients with advanced bronchogenic carcinoma were used in the study and were randomized into different treatment groups. A series of immunological tests, such as the skin tests for four antigens, the total T, active T and B cell assays, and the PHA stimulation of lymphocytes were done routinely on all patients. The immunological profile was obtained following the BCG and RT therapy.

To date, 106 patients have been entered on the BCG immunotherapy protocol, 23 were dropped due to either patient

refusal or other reasons specified in the protocol, 40 were treated with BCG plus radiotherapy (BCG-RT) and 43 were treated with radiotherapy alone (RT).

The median survival time of the BCG-RT group is 286.3 days, while for the RT group it is 361.9 days. The difference is not significant statistically. Immunological tests done have been analyzed with no significant findings to this date. The results of this study are being tabulated and will be published soon.

128. NON-SPECIFIC IMMUNOSTIMULATION IN TREATMENT OF BRONCHOGENIC CARCINOMA

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Since the beginning of this protocol, we have entered forty-two patients. All had carcinoma of the lung, Stage I or II, and all received nonspecific immunostimulation using BCG Glaxo in the pleural cavity. There were no complications due to the installation of BCG and all patients left the hospital at regular intervals. They were followed closely in the Outpatient Clinic.

Thus far there have been 12 deaths. The longest survival has been 36 months and the shortest survival has been four months.

The installation of Glaxo BCG into the pleural cavity is very simple. We have used either an intracath plastic needle or previously placed chest tubes. None of the patients developed any high fever or untoward reaction to the BCG.

We are continuing to treat new patients with this protocol.

129. CANCER CHEMOTHERAPY AND IMMUNOTHERAPY PROTOCOLS, SWOG 7635, 7703, 7704

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SWOG 7703 - The purpose of this study is to compare the effectiveness of radiation therapy plus BCNU, radiation therapy plus DTIC, and radiation therapy plus procarbazine for remission induction, duration of remission, and survival in patients with malignant gliomas of the brain. A total of 6 patients from this institution were entered into this study. The study was activated in March of 1977 and remains open. The response rate for procarbazine is 18 percent, BCNU 33 percent, DTIC 30 percent, and there is no difference in survival.

SWOG 7704 - To compare the effectiveness of three intermittent plus chemotherapy combinations VMCP plus VCAP vs. VMCP plus VBAP vs. MP for induction of remission in previously untreated patients with multiple myeloma. Results will also be compared with other combination chemotherapy treatments in previous SWOG studies, especially VMCP treatment in SWOG 7418 and previous studies of MP combinations. For patients proven to have at least a 75 percent tumor regression after induction, to compare the value of 12 months of chemoimmunotherapy maintenance VMCP plus levamisole in comparison to VMCP alone. To establish baseline and serial data on immunologic status in these patient groups. This study began in March of 1977 and closed in August of 1979. A total of 8 patients from this institution were entered into the study. The response rates for the three regimens are 47 percent for VMCP plus VCAP, 41 percent for VMCP plus VBAP, and 38 percent for MP, no significant difference. The number of patients relapsed was smallest in the VMCP plus VBAP group. There is a difference in survival between the VMCP plus VBAP group vs.the MP group at the 5 percent level. This favors VMCP plus VBAP.

130. TUMORS OF THE NERVOUS SYSTEM

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A clinical study of intratumoral chemotherapy using 8-azaguanine compares the effect of this method given in combination with systemic CCNU after surgery and radiotherapy with the effect of adjuvant chemotherapy with CCNU given alone. In a pilot study, our methods have increased the mean survival time to approximately twice that reported by other centers using CCNU alone after surgery and radiotherapy. Dosage of therapeutic agents used for local intrathecal chemotherapy is being monitored in a primate model for chronic intrathecal chemotherapy. Our immunologic approach is currently embodied in a Phase I study of treatment with thymosin. Based on in vitro studies with thymosin in our gliomas patients we have completed a Phase I study comparing the in vitro effects of thymosin V and thymosin alpha 1 in 14 patients. Further work has been stopped due to administrative interference.

131. INTENSIVE CHEMOTHERAPY AND IMMUNOTHERAPY IN NEUROBLASTOMA STAGES III AND IV - PHASE II STUDY

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HISTOLOGIC TYPE AND CLINICAL STAGE: Neuroblastoma - All paediatric patients with stage III and stage IV neuroblastoma are eligible for study.

TREATMENT MODALITIES: Vincristine, Cyclophosphamide, Adriamycin and Imidazole Carboxamide (DTIC). Transfer factor prepared from one of the parents if tumor immunity can be demonstrated in the parents using a migration inhibitor factor (MIF) assay.

PROTOCOL OUTLINE: The trial will open to all patients with stage III and stage IV neuroblastoma, who have not received any previous chemotherapy. The chemotherapy will be given as follows - Vincristine 1.5mg/m2 IV on days 1 and 5, Cyclophosphamide 600mg/m2 IV on day 1, Adriamycin 50mg/m2 IV on day 3, DTIC 200mg/m2 IV on days 1 to 5. This regimen will be repeated every 29 days. When remission is achieved transfer factor will be given on day 22 of each cycle. Observations during study will be - symptoms and physical findings, documenting size of main lesion; urine catecholamines every eight weeks until normal, than every twelve weeks; skeletal survey every eight weeks till clear, then every twelve weeks; bone marrow aspiration every 8 weeks (if positive initially) until negative, then as indicated clinically. MIF assays to assess anti-tumor immunity will be performed every 12 weeks in the patients treated with transfer factor to document any alterations in tumor immunity. Patients with progressive disease will be removed from study. Patients showing a partial response or static disease will remain on study until progressive disease occurs. Patients who demonstrate a complete response will remain on therapy for 24 months.

PROGRESS: Twenty patients entered on study, 13 patients died, 7 alive. Median survival time - 22 months. Study will continue, and it is planned to enter 4 or 5 patients per year.

132. HUMAN SPECIFIC IGG ATTACHED TO RUBIDOMYCIN AS A NEW APPROACH TO TREATMENT OF METASTIC NEUROBLASTOMA

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OBJECTIVE: Considering that stage IV of neuroblastoma has normally a fatal course with standard treatment, it is proposed to attempt a new type of therapeutical approach. It consists in the use of human specific IgG attached to rubidomycin that has shown in vitro a 100% cytocidal effect on the neuroblastoma cells.

APPROACH: All the children with neuroblastoma, stages III and IV, sent to the Pediatric Oncology Center of the Pediatric Department of Rome University are included in the present research.

Neuroblastoma cells are being cultured directly from the tumor mass or from the bone marrow of the patient. They are then irradiated with 10,000 r and administered twice to one of the parents, chosen by his/her immunological response (MLR) to the tumor cells. After two weeks, specific IgG are obtained from the plasma of the parent and attached to rubidomycin. The substance is then administered to the child. The effect of the therapy is assayed before and after the treatment.

133. HUMAN NEUROBLASTOMA CELL SURFACE ANTIGENS

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There is a well recognized need to improve the therapy of childhood neuroblastoma. Our studies aim to characterize neuroblastoma cell surface antigens (CSA) by serologic and biochemical criteria and then to develop immunologic tests and immunotherapy utilizing them. To characterize CSA, we use cultured neuroblastoma cells and non-cultured tumors and tissues, monoclonal antibodies produced by lymphocyte hybridomas, rabbit and human antisera, the radioiodinated protein A assay for cell surface bound antibodies, and isoelectric focusing/polyacrylamide gel electrophoretic analysis of immunoprecipitated radiolabeled CSA. Emphasis will be placed upon identifying CSA which are specific for neuroblastoma cells or for fetal cells. Immunologic tests will include quantitation released (shed) CSA and determination of CSA phenotype. These tests may provide means of classifying tumors and of predicting and monitoring the response of neuroblastoma to therapy. Some CSA may serve as targets for immunologically specific cytotoxic or cytostatic reactions providing considerable therapeutic potential. Clinical studies of children with neuroblastoma and others will determine the incidence of antibody responses to CSA as an indicator of immunogenicity, and in vitro and in vivo model studies will determine the ability of antibodies to affect neuroblastoma cell survival and growth. Development of immunologic tests and immunotherapy should significantly contribute to our long-term aim of achieving optimal therapy for children with neuroblastoma.

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134. IMMUNO-CHEMOTHERAPY OF MALIGNANT BRAIN TUMORS

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OBJECTIVES: Objectives of the study are: a) To determine in a controlled, prospective and randomized study if the combination of radiotherapy (RT), chemotherapy (BCNU and CCNU) and immunotherapy with BCG is superior to treatment with radiotherapy and chemotherapy (BCNU or CCNU) alone in terms of response rate, prolongation of survival time and improvement of quality of life. b) To determine relationships between immunologic parameters, performance status, response rate and survival.

APPROACH: Patients are stratified into two groups: Category A: patients with operable malignant brain tumors; Category B: patients with inoperable malignant brain tumors.

Patients of Category A are randomized to RT plus BCNU (control arm) or RT, BCNU and BCG; patients of Category B to RT plus CCNU (control arm) or RT, CCNU and BCG.

PROGRESS: Since July 1, 1979, (beginning of the study) we have studied 8 patients ranging from 31 to 68 years of age, 6 males and 2 females. Five patients underwent surgery for removal of the tumoral mass (Category A). Three were randomized to the control group and two to adjuvant immunotherapy. Three patients were diagnosed as inoperable (Category B) and thus randomized: 2 to the control arm, 1 to the adjuvant immunotherapy. During treatment 4 patients died, 2 of intervening causes and 2 of tumor regrowth.

Due to the limited number of patients entered into the study to date it is presently impossible to draw conclusions relative to the research objectives.

135. BRAIN TUMOR IMMUNOTHERAPY (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

136. ANTIGENIC COMPONENTS OF HUMAN PROSTATIC ADENOCARCINOMA

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Immunological approaches to human prostate cancer depend upon the existence of cell-surface antigens capable of evoking a specific immunological response. The purpose of our study is to determine whether patients with prostatic cancers develop an immunological response to antigens unique to their tumor cell surfaces. In addition to providing information about the fundamental cellular changes underlying malignant transformation, these studies have the immediate practical goals of providing the means to assess the immunological response of patients to their tumors during the course of disease both with and without treatment; of opening avenues for manipulating the immunological response in favor of the tumor-bearing host; and of developing immunological methods for the earlier and more accurate detection of prostatic cancers.

Carcinoma of the prostate provides an excellent model for studying cancer immunology in man because of its relatively high prevalence and chronic nature. It is a tumor whose progress can be slowed down by surgery and, in some cases, by hormonal therapy or chemotherapy. Because patients with prostatic carcinoma are in relatively good health during much of the course of their disease, they have an opportunity for developing an immune response to the tumors.

We plan to continue our studies in prostatic carcinoma by evaluating the specific immunologic response of patients to their own tumors. Cell-mediated as well as humoral responses will be assessed by several new approaches. These include in vivo and in vitro cytotoxicity assays as well as biochemical studies of prostatic tumor specific antigen. These investigations should provide a rational basis for immunotherapy aimed at modifying the response to this cancer in man.

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137. EVALUATION OF SURGERY, RADIOTHERAPY AND IMMUNOTHERAPY IN STAGE B AND C CARCINOMA OF THE PROSTATE

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The objectives of this report are to compare the effectiveness of surgery plus immunotherapy against surgery plus radiotherapy in the local control of prostatic carcinoma in clinical Stages B and C with positive nodes. We also want to compare the effectiveness of two modalities of local radiotherapy (implant and external beam), as well as the meaning of positive biopsies after radiotherapy, and to correlate the assays of immunocompetence with the clinical course of the various treatment modalities.

The research plan is to randomize patients with Stage B to surgery plus immunotherapy, or surgery plus radiotherapy. If the second randomization was chosen, patients would recieve surgery alone if the nodes in the pelvis were negative, or surgery and post-operative radiotherapy if these nodes were positive. For Stage C, the same randomization (surgery plus immunotherapy vs. surgery plus radiotherapy) was done. However, if surgery plus radiotherapy was chosen, further randomization will be done to receive either seeds to the prostate with iodine 125 or external beam.

Unfortunately, our accrual of cases has been very small. Approximately 9 cases have been accrued so far. No case has been found to be suitable for the implant with iodine 125 seeds. Hence, no real findings are available so far.

The protocol does not require any funding; it is a tool to allow the patients with carcinoma of the prostate to receive a uniform treatment at the University of Texas Health Science Center at San Antonio. Hence, although the accrual has been

very disappointing, we still would like to keep it open, hoping that eventually, in the future, the accrual will increase.

No new developments have been reported in the literature during the last year to show that our protocol needs to be changed.

138. USE OF THE CELL SEPARATOR IN THE TREATMENT OF SELECTED CANCER PATIENTS

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APPROACH: Human neuroblastomas, melanomas, or hypernephromas are cultured and farmed for up to six generations. Tumor cells irradiated with 10,000 rads are injected into informed patient. Samples are later tested against the relative tissue culture, and also against the normal leukocytes of the donor of the tumor. This use of one haplotype-identical volunteer results in few reactions against the normal leukocytes of the patient. The reaction against the tumor cells is assayed to see whether it is mainly humoral or cellular: if cellular, the patient may be treated with irradiated lymphocytes harvested from the volunteer, or with transfer factor prepared from such lymphocytes, or indeed with extracts of RNA similarly prepared. If humoral, we harvest large quantities of plasma from the volunteer, process it over cold alcohol (COHN) to isolate the gammaglobulin fraction, to which either chlorambucil or rubidomycin is conjugated. Checks are made for sterility and pyrogenicity before use.

PROGRESS: Over 120 human volunteers have been immunized with no side effects so far, and in almost every case a usable immune reaction against the tumor. Therapy with immune cells has only been attempted in eight patients, all of whom were 'unblocked' prior to receiving the cells, and in all cases an objective regression of tumor was observed, but was nearly always temporary. Attempts to educate the leukocytes of the patient with extracts from the volunteer have so far failed (? any recipient clones).

Chemoimmunotherapy with 'tumor-specific antibodies' coupled to cytotoxic drugs has now been used in 10 Stage I patients, achieving impressive objective regression of tumor, but thus far transient up to 15 months.

139. PREVENTIVE VACCINATION AGAINST CANCER

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To date, 127 patients with advanced cancer have been treated with double vaccination, active and passive. The patient is injected with tumoral antigen and receives blood transfusions from a donor hyperimmunized with the same antigen. In the case of patient and donor of unmatched blood type, only plasma and leukocytes are used. Hematologic tests on such injected donors, using the same antigen as that used for the patient, show a great increase in the IMG as well as in the immune cells programmed to destroy cancerous cells. Specific double immunization can destroy tumors in cancer patients, and more easily, post-surgical residual or recurring tumors. We have also observed that after chemotherapy or radiotherapy the destructive activity of the immunotherapy against malignant tumors is facilitated, being much more aggresive.

Of the 127 cancer patients treated, all of whom statistically should have died, 69 are dead, 12 for reasons unrelated to cancer. Of the 58 patients who are alive, 26 have been treated within the last year and no results are available as yet. The remaining 32 patients have survived 1-6 years.

To date, 320 healthy persons have been vaccinated; all come from families of cancer patients and are therefore considered to be at high-risk of developing cancer. Among the vaccinated persons, there is no report of development of cancer within the last 6 years, nor has any accident occurred in the use of this vaccine either in patients or in healthy persons. (Translated)

140. CANCER CHEMOTHERAPY OF HEMATOLOGIC MALIGNANCIES

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PURPOSE: To evaluate chemotherapy and chemo-immunotherapy regimens in the treatment of human malignancies.

MATERIALS AND METHODS: Patients were treated according to tumor type on various protocols of the Southwest Oncology Group. Currently, there are 42 active protocols being used at this institution. In the past year, 22 patients have been registered on protocols.

RESULT: Four patients with malignant lymphomas were treated. Three achieved complete response and remain in complete remission. One achieved a partial response and has since relapsed. Two patients with malignant melanoma were treated. One had a complete response, but then refused further therapy until he had recurrence of his disease. Three patients suffering from malignancies of the bladder (1), salivary gland (1), lung (1) were treated and all achieved partial responses. One patient each with acute leukemia, malignancy of prostate, head and neck malignancy were treated and had no responses. A number of patients have been treated on Phase II studies including 4 lung, 2 melanoma, 1 each myeloma and lymphoma colon, and acute leukemia and none of these patients have responded to therapy. There has been no untoward toxicity during the past 12 months in any of our patients and we will continue to place as many patients on protocol as possible.

141. CHEMOTHERAPY OF ADVANCED MALIGNANCIES - SWOG 7524

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Chemotherapy of Advanced Malignancies - The objectives of the protocols herein contained are to improve the results of treatment for patients with non-Hodgkin's lymphomas and squamous carcinoma of the head and neck. In the former situation a prospective, randomized comparison of two different combination chemotherapy regimens and one chemoimmunotherapy program is to be carried out. In the latter, patients are to be randomized between methotrexate alone, a standard form of treatment, and a three-drug combination combining Methotrexate and 2 other active drugs. Comparisons will be made based on response rate, toxicity and survival data. As soon as a conclusion can be reached regarding superiority of a particular regimen, these studies will be closed.

SWOG 7524 - To compare the effectiveness of chemotherapy alone (adriamycin-cyclophosphamide) vs. chemoimmunotherapy (adriamycin-cyclophosphamide plus BCG) for remission induction in patients with Stage III and IV ovarian and endometrial carcinoma who have had no previous cytotoxic chemotherapy. To test the effectiveness of continued chemoimmunotherapy vs. chemotherapy in maintaining complete remissions (documented) achieved during the initial 12 month induction therapy. To test the effectiveness of continued chemoimmunotherapy vs. chemotherapy in inducing complete remissions or maintaining partial remissions in patients with occult disease at the time of restaging for complete response or in patients achieving only partial clinical remission during the initial 12 month induction therapy. This study was activated in October of 1974 and closed in October of 1976. All patients from this center were entered into the study. 154 were entered group-wide. The data showed that adriamycin-cyclophosphamide plus BCG was better than adriamycin-cyclophosphamide alone in terms of actuarial survival. No further case entries are anticipated.

I. OTHER CLINICAL IMMUNOTHERAPY STUDIES AND BROAD PROGRAMS

142. TRANSFER FACTOR AND ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY

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OBJECTIVE: To investigate the effect of transfer factor (TF) therapy on antibody-dependent cellular cytotoxicity in humans.

APPROACH The role of antibody-dependent cellular cytotoxicity (ADCC) is evident in the defense against tumors, viral infections etc. and under some immunopathologic conditions like autoimmune hemolysis. The enhancing effect of TF

therapy on direct cytotoxicity has been shown in osteosarcoma (Levin et al.).

PROGRESS: During the treatment of ten patients with tumors or immunodeficiency we have observed that decreased ADCC increases following just one day and persists at a high level over a period of more than a week. The phenomenon is not connected with alterations in the peripheral number of Fc receptor bearing cells. Normal indices do not change significantly after TF therapy. This modulating effect of TF on ADCC in vivo may contribute to its therapeutical benefits.

143. TRANSFER FACTOR IMMUNOTHERAPY IN CANCER - CLINICAL APPLICATIONS AND BASIC PROPERTIES

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Progress on this project has been achieved in 2 major areas: (1) Fractionation and characterization of transfer factor (TF). (2) Detection of reactivity to tumor antigens in cancer patients by the leukocyte adherence inhibition (LAI) test, and by a new approach, the antigen binding cell assay.

Transfer factor activity measured by the transference of delayed type skin test reactivity from immunized donors (humans or calves) has been characterized by chemical separation (high pressure liquid chromatography and isoelectric focusing) and enzymatic treatment (alkaline phosphatase, pronase, proteinase K, and phosphodiesterase I and II). Results suggest TF separates as a dislinite entity of low molecular weight and strongly negative charge and has both peptide(s) and nucleotides as structural components. Melanoma neuroblastomic and leukocyte adherence inhibition test used to evaluate immunifying patients with squamous cell carcinoma of the head and neck and families with a high incidence of melanoma has detected higher levels of reactivity within specific tumor groups to their appropriate tumor extract. The mechanism involved in this assay includes reactions by multiple cell types, and serum factors from immune donors which can arm nonimmune cells to be reactive to specific tumor extracts. The antigen binding cell assay - which measures formation of rosettes between immune cells and tumor antigen-conjugated red cells - has detected an increased percentage of mononuclear cells which can bind squamous carcinoma antigen in patients with advanced primary tumor but not in patients with recurrence or in controls.

144. EVALUATION OF A NEW IMMUNOSTIMULATORY DRUG IN HUMANS

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A new immunostimulatory drug termed Bestatin, which is a fermentation product of Streptomyces olivoreticuli, is the subject of a phase I study in patients with advanced cancer. The patients receive the drug orally at a dose of 10 mg three times daily. Apart from regular examinations of liver and kidney functions the patients peripheral lymphocytes are examined before and during Bestatin treatment with respect to the cellular composition (T- and B-cells), reactivity to PPD and PHA in vitro and capacity to spontaneously kill allogeneic tumor cells in vitro. So far, no undue side effects of the drug have been observed and there is evidence that Bestatin treatment increases the proportion of T-cell and activates NK cells.

145. IMMUNOPROPHYLAXIS OF CANCER WITH BCG VACCINE

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The aim of this study is to determine retrospectively the long-term effect of BCG vaccination in black children vaccinated in the first week after birth and followed up for 20 years. Death records of children born at Cook County Hospital are being examined. Cross-checking of records for the entire United States for any deaths among the study population is being made to prevent loss of data.

146. A PROSPECTIVE STUDY OF CANCER RISK IN TUBERCULOSIS

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Interest in the hypothesized association between tuberculosis and cancer has been intensified by studies demonstrating that tubercle bacilli (i.e.BCG) potentiate host immune responses against cancer challenge. Recent experiments in man suggest that BCG acts not only to evoke non-specific cell-mediated immunity but also to increase host resistance against specific tumors such as melanoma, sarcoma and breast cancer.

In view of the above, a type of natural experiment in cancer prevention is underway. A large cohort of tuberculosis patients is being followed prospectively through time to death or suuvival as of December 31, 1974. The outcome variables of interest are cancer mortality and morbidity. Observed cancer risks will be compared with those of the general population similar in age, sex and race. Additional analyses will focus upon cancer risk according to clinial severity of tuberculosis, with adjustment for smoking habit. Consideration will also be given to cancer risks among close family members as a further test of the antitumor immunogenicity of exposure to tuberculosis.

The prospective design of this study is relatively free of biases inherent in earlier efforts. This will also make possible the study of the association between tuberculosis and all types of cancer. A significant relationship between the two could suggest the possibility of BCG or tuberculin vaccination as a cancer preventive.

147. STUDY OF BACTERIAL COMPONENT IMMUNOTHERAPY IN CANCER

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The objectives of this research proposal are to determine through clinical and laboratory studies the toxicity, immunological effects, efficacy, mechanism of action and clinical role of chemically defined bacterial component immunotherapy in human malignant disease.

These objectives will be approached by detailed clinical Phase I and II studies of purified components used as single agents. The particular agents included in this current study are: aqueous suspension of M. smegmatis CWS; aqueous suspension of M. smegmatis CWS combined with trehalose mycolate; and oil attached M. smegmatis CWS and trehalose mycolate. We will also study the endotoxin from the Re mutant Salmonella typhimurium.

All of these materials will be used intravenously.

Phase I studies will be done in patients with advanced lung cancer, sarcoma, melanoma and hypernephroma. Phase II studies will be done mainly in patients with malignant melanoma and hypernephroma. We have observed response in these patients and consider these diseases 'indicator systems' of active immunotherapeutic agents.

Concurrently, immune testing will be performd to define the immunological effects of these agents, and to attempt to identify patients who have a high probability of responding to these agents.

148. TUMOR IMMUNOLOGY AND IMMUNE SURVEILLANCE

Poplack, D. G., Reaman, G. H., Blatt, J., Trigg, M., Blaese, R. M., Broder, S., Pichler, W., Smyth, J., Donlon, J., Bollum, F., Ladisch, S. K., Callahan, T., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Pediatric Oncology Branch, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

In this project, mechanisms of normal cellular immunity and tumor immunology are studied. Immunological surveillance of cancer patients and the effects of chemotherapy and immunotherapy on the immune response are explored. We are characterizing acute lymphocytic leukemia according to the immunological subtypes of lymphoblasts and the capacity of lymphoblasts to express immunoregulatory helper and/or suppressor functions in vitro. Possible correlation between lymphoblast cell surface markers with various biochemical characteristics is being pursued. Particular emphasis is placed on immunologic study of

the monocyte-macrophage system (including development of new clinical assays of human monocyte function) in malignancies and immunodeficiency diseases, in collagen diseases, and in the histiocytoses. We are also investigating serum suppressors of cellular immunity in cancer patients. The therapeutic and diagnostic potential of anti-leukemia antisera and leukemia-specific hybridoma antibodies is being evaluated in vitro and in vivo.

BIBLIOGRAPHIC REFERENCES: Blaese, R.M., Poplack, D.G., and Muchmore, A.V.: The mononuclear phagocyte system: Role in expression of immunocompetence in neonatal and adult life. Pediatrics 64 (Suppl): 829-833, 1979. Reaman, G.H., Lum, L.G., and Poplack, D.G.: The detection of Fc-IgA receptors on T and non-T, non-B acute leukemic lymphoblasts. Cell. Immunol. 52: 218-222, 1980.

149. IMMUNOCOMPETENCE OF CANCER PATIENTS

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The overall objectives of this proposal are to evaluate the non-tumor-specific immune reactivity of cancer patients in order to determine the effect of malignancy itself and cancer therapy upon the patients' immune resistance. A major portion of this proposal has focused on the macrophage and PMN. A reliable method of obtaining the human macrophage has been developed and we are continuing to investigate the effect of neoplasia and cancer therapy upon macrophage and polymorphonuclear leukocyte function, and the relationship of the macrophage and PMN to effector lymphocytes, and are seeking to determine the effect of non-specific immunologic stimulants, such as BCG and interferon on macrophage and PMN functions.

Methodologies to be employed are as follows: Macrophage and PMN cytotoxicity, subcelluler fractionations and in vivo models utilizing immunosuppressed hamsters.

We have previously learned from this project that there is an immune deficit associated with progressive neoplasia. Our results indicate that there is an excellent correlation between DCHR and DNCB and the clinical extent of malignancy. In vitro results indicate that PMNs such as lymphocytes and macrophages can selectively destroy tumor cells both in vitro and in vivo. Continued efforts in purification of isolated PMN cytoxic factors are underway.

This program is highly relevant to the treatment of patients, since a clearer insight into the mechanism of tumor cell destruction by the macrophage and PMN may lead to a better understanding of immune reactivity to tumor cells and thus a more effective means of treatment of human neoplasia may be possible.

150. ONCOLOGY STUDIES - EASTERN COOPERATIVE ONCOLOGY GROUP

Stolbach, L. L., Huberman, M., Gandbhir, L., Amick, C., Kaiser, C. W., Patel, V. C., Reid, G. M., Choi, O. S., Tufts University, Boston Campus, School of Medicine, Dept. of Medicine, *136 Harrison Ave., Boston, Massachusetts,* 02111, United States of America

The Tufts Oncology Group is submitting this application for continued support of activities aimed at improving the diagnosis and therapy of patients with cancer. The major aim of the participating members of the ECOG is to supply clinical material for ECOG protocol evaluation of chemotherapeutic and immunotherapeutic agents as well as radiation therapy used singly or in combination in patients with advanced malignancy. In addition, in selected tumors therapy will be evaluated following optimal surgical therapy. The program represents an interdisciplinary approach to the patients with malignancy, combining the disciplines of medical oncology, radiation therapy, surgical oncology, pathology and immunotherapy.

151. IMMUNOLOGICAL ASPECTS OF CANCER PROGNOSIS

Hersh, E. M., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas,* 77030, United States of America

In this project, immunological reactivity of cancer patients will be studied before treatment and during chemotherapy and immunotherapy. The objective of the study will be to establish the relationship between immunocompetence and prognosis, and to accurately evaluate the immunological effects of chemotherapy and immunotherapy. The least immunosuppressive chemotherapy and the most immunopotentiating immunotherapy will be sought. The immunological tests to be evaluated will include skin tests with recall antigens, a lymphokine skin test, and a variety of cell-mediated immunity studies in vitro, including the following: tests of macrophage function including 14C glucosamine incorporation, phagocytosis and macrophage cytotoxicity, lymphocyte cytotoxicity induced by allogeneic stimulation, and antibody-dependent cell-mediated cytotoxicity. The disease categories to be studied will include melanoma stage III and IV, breast cancer stage IV, lung cancer stages I-IV, and leukemia. Immunotherapies to be studied include BCG, C. parvum and MER. The potential benefit will be improved prognostication for the cancer patient and the selection of better therapies.

152. SOUTHWEST ONCOLOGY GROUP

McKelvey, E. M., Bodey, G. P., Burgess, M. A., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept. of Developmental Therapeutics, *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas,* 77030, United States of America

The objective of this research is the development of more effective treatment for patients with a wide variety of solid tumor malignant diseases. Toward this end, we will continue our activities within the Southwest Oncology Group, participating in active studies, conducting pilot programs relating to future group protocols, providing information on new chemotherapeutic and immunotherapeutic agents and lending our expertise in the design, implementation and analysis of SWOG protocols. Close interdisciplinary interaction will be maintained between investigators at M. D. Anderson and with those from other SWOG institutions so that other practical and effective multimodal programs may be developed within this group. This proposal reflects the continued interest and participation of a number of investigators having broad clnical experience in cancer therapy who have made many significant contributions to the Southwest Oncology Group.

BIBLIOGRAPHIC REFERENCES: Herman, T. S., Hammond, N., Jones, S. E., Butler, J. J., Byrne, G. E., Jr., and McKelvey, E. M.: Involvement of the central nervous system by non-Hodgkin's lymphoma: The Southwest Oncology Group Experience. Cancer 43:390-397, 1979. McKelvey, E. M., Fisher, R. J., and Haskins, C.: Clinical features of malignant lymphoma. Proceedings of the American Society of Clinical Oncology (C-380) 20:383, 1979.

153. IMMUNOTHERAPY OF HUMAN CANCER

Morton, D. L., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Section, *16111 Plummer St., Sepulveda, California,* 91343, United States of America

The specific goals of this proposed research are as follows: 1. To define and characterize tumor-associated antigens of lung carcinoma. 2. To isolate and purify tumor-associated antigens and to use them to develop a radioimmunoassay for the detection of tumor-associated antigens in the sera and urine of lung cancer patients. 3. To evaluate in vitro assays of immune function to determine the usefulness of a particular immunologic assay and of a particular lung cancer antigen to accurately reflect total body burden of tumor.

Lung carcinoma antigens will be isolated in soluble form from abstracts of lung carcinoma tumor cells or spent medium of tissue culture cells. Antigens will be purified using fractional precipitation with ammonium sulfate, KBr flotation and column chromatograhy. Assays employed will be leukocyte migration inhibition and complement fixation for cellular immunity and isotope binding and immunofluorescence for serologic tests.

A group of lung carcinoma associated antigens has been identified. Antibodies directed against these antigens have been found in 50% of lung cancer patients tested. These antibodies have been shown to be not directed against HLA or other normal transplantation antigens, oncofetal antigens or tumor-associated antigens associated with some other histologic types of cancer.

This research program is highly relevant to the clinical situation. If new diagnostic and prognostic indicators of human lung carcinoma are discovered, the disease may be diagnosed in

its early stages and chances of a cure for these patients will be markedly increased.

154. CANCER IMMUNOTHERAPY

Hirshaut, Y., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

Independently and not as an agent to the Government, the Contractor shall exert its best efforts to evaluate selected immunotherapeutic agents for acute and chronic toxicity, maximum tolerated dose, effects or immune status, optimum immunostimulating dose, optimum immunostimulating schedule, optimum immunostimulating route of administration and, if appropriate, therapeutic effectiveness in selected cancer patients. All studies shall be performed in accordance with clinical protocols which have first been approved by the Project Officer. Forty patients are required for evaluation of each agent and 80 patients are required for this contract period. Immunotherapeutic agents shall be selected in consultation with the Project Officer.

155. HYBRIDOMA DERIVED MONOCLONAL ANTIBODY FOR EXPERIMENTAL AND CLINICAL CANCER THERAPY

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No summary has been provided to the Smithsonian Science Information Exchange.

156. IMMUNOTHERAPY FOR DISSEMINATED MALIGNANT NEOPLASMS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

157. TRANSFER FACTOR IN CANCER PATIENTS

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This is part of a broader project. A summary of this subproject is not available.

158. CHEMOTHERAPY AND IMMUNOTHERAPY OF MYCOSIS FUNGOIDES

Van Scott, E. J., Temple University, School of Medicine, Dept. of Dermatology, *3322 Broad St., Philadelphia, Pennsylvania*, 19122, United States of America

This is part of a broader project. A summary of this subproject is not available.

159. SPECIFIC IMMUNIZATION WITH CANCER CELL VACCINES

Oettgen, H. F., Memorial Hospital for Cancer & Allied Diseases, *1275 York Ave., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

160. CANCER THERAPY WITH IMMUNOPOTENTIATORS - PHASE II AND III TRIALS

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This is part of a broader project. A summary of this subproject is not available.

161. TREATMENT OF CANCER WITH IMMUNOMODULATORS

Hirshaut, Y., Memorial Hospital for Cancer & Allied Diseases, *1275 York Ave., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

162. THERAPEUTIC IMMUNOMODULATION (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

163. IMMUNOTHERAPY OF PATIENTS WITH CANCER

Jewell, W. R., University of Kansas, Medical Center, School of Medicine Dept. of Surgery, *39th St. & Rainbow Blvd., Kansas City, Kansas*, 66103, United States of America

This is part of a broader project. A summary of this subproject is not available.

164. CLINICAL IMMUNOTHERAPY

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This is part of a broader project. A summary of this subproject is not available.

165. IMMUNOTHERAPY (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

II. PRECLINICAL ASPECTS OF CANCER IMMUNOTHERAPY

A. IMMUNOPOTENTIATORS

1. BCG

166. COMPARATIVE ANTITUMOR ACTIVITY OF BCG SUBSTRAINS

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OBJECTIVE: To compare, in animal models, the antitumor activity of BCG substrains grown under identical conditions.

APPROACH: Ten BCG substrains were included in the study and their immunoprophylactic and immunotherapeutic activities were compared in three systems of transplantable tumors: the Ehrlich carcinoma in its ascitic form, the L1210 lymphoid leukemia and the B16 melanoma. Mice used were, respectively, CF1, C57BL/6 x DBA/2 hybrids (B6D2F1) and C57BL/6. BCG doses used were based on the number of CFU for each substrain and, in each experiment, the treated animals were given comparable numbers of CFU. Comparable numbers of live tumor cells were also used for a given experiment. Antitumor activity was evaluated by the survival rates following tumor graft and all results are based on statistical analyses.

PROGRESS: In all of the three systems of transplantable tumors used, the immunoprophylactic activities detected proved to be greater than the immunotherapeutic ones, and this was true for all substrains. Higher doses of BCG did not induce better antitumor activity for all substrains, some of which were equally active at low and high dosages. Differences in antitumor activity were detected between the substrains which could be classified in three categories of activity: high, moderate and inactive. A given substrain was not found to be equally active in all the three systems of transplantable tumors used.

167. ANTI-TUMORAL EFFECT OF CRYOSURGERY

Serrou, B., Hopital St. Eloi, Laboratoire d'Immunopharmacologie des Tumeurs, *B.P. 5054, 34033 Montpellier, France*

The aim of this project is to evaluate the anti-tumoral effect of cryosurgery on tumor growth and the immune response, when used alone or in combination with non-specific immunotherapy in the form of BCG. Two stages of investigation are involved. The first stage will consist of establishing cryosurgery techniques from the point of view of choice of electrode and the measure of intratumoral temperatures so as to determine the freezing/thawing cycle which will lead to total destruction of tumor tissue, avoiding damage to healthy tissue, thus deriving the number and frequency of cryosurgical treatments required.

The second stage involves the use of the Lewis tumor model which will be transplanted into C57BL/6 mice. This model will permit us to examine the effect of cryosurgery on tumor growth and the evolution of pulmonary metastases. Once such an effect has been evaluated cryosurgery will be associated with different modalities, i.e., BCG therapy, following the same pattern that we have already used after radiotherapy. This will enable us to ascertain whether cryosurgery potentiates the effects of non-specific immunotherapy. This appears to us to be an important aspect of the study for it has far-reaching implications in the treatment of human tumors such as malignant melanoma. This work is in progress and is at the stage of refining the cryosurgery technique which in the coming few months will allow us to examine its effects on the chosen tumor model.

168. ANTITUMOR ACTIVITY OF BCG, C. PARVUM AND GLUCAN

Megirian, R., Loose, L. D., Union University, Albany Medical College, School of Medicine, Dept. of Pharmacol. & Exptl. Therapeutics, *47 New Scotland Ave., Albany, New York,* 12208, United States of America

The purpose of this project is to compare the antitumor effects of 3 known stimulants of macrophages (BCG, C. parvum and glucan) in several tumor models (syngeneic and allogeneic) in order to more precisely determine, with respect to nonspecific macrophage activity, (a) how each stimulant acts (similarities and differences) to induce tumor resistance, (b) which agent is the most effective, and (c) by what protocol to produce maximum antitumor effects. The production of activated macrophages capable of inducing tumor remission produces humoral (mediators) and macrophage cellular (metabolic) changes. By examining basic similarities and differences in the possible sites and intensity of action of BCG, C. parvum and glucan against strain- and non-strain-specific tumor models in 2 different species (rats and mice), we will be able to better understand the essential response(s) elicited by each of the 3 stimulants and the tumor models selected for study. The aspecific opsonin (also called HRF), which does play an apparent role in neoplasia, will be followed, as will selected macrophage metabolic activities (O2 uptake, protein synthesis, enzyme activities) in order to assess what role humoral and metabolic changes play in the remission of these tumors by this nonspecific means. We will, separately, manipulate the levels of the aspecific opsonin (HRF) and cellular metabolic activities (with trypan blue, hydrocortisone, methyl palmitate) of macrophages to help determine sites of action of each agent. Thus, we will determine on a comparative basis how, by what means, and to what degree each stimulant can act to induce increased resistance with each tumor model. This has obvious clinical significance. The present ongoing studies have begun to supply some very interesting and highly worthwhile results during the past few months.

BIBLIOGRAPHIC REFERENCES: Astry, C. L., Megirian, R., Sosler, R. J. and Loose, L.: The effect of Corynebacterium parvum (C. parvum) on peritoneal macrophage function in BDF1 mice. J. Reticuloendothel. Soc. 24: 4a, 1978. Megirian, R., Astry, C. L. and Loose, L.: Antitumor activity of Corynebacterium parvum (C. parvum) and trypan blue in mice bearing the P388 tumor. J. Reticuloendothel. Soc. 24: 22a, 1978.

169. ANTITUMOR ACTIVE COMPONENTS OF BCG CELL WALLS

Gray, G. R., University of Minnesota, Minneapolis Campus, School of Chemistry, Dept. of Chemistry, *105 Morrill Hall, Minneapolis, Minnesota,* 55455, United States of America

The objective of this research is to identify the structural features of mycolic acids and the mycobacterial cell wall lipopolysaccharide-mucopeptide complex that are necessary for antitumor activity in order, hopefully, to provide more active and less toxic immunotherapeutic agents for use in the treatment of cancer. Isolation procedures developed in this laboratory have provided, for the first time, mycolic acids as structurally pure molecular entities, which makes possible a thorough examination of their structures and the structural basis for their activity.

BIBLIOGRAPHIC REFERENCES: The Major Mycolic Acids of Mycobacterium smegmatis. Characterization of Their Homologous Series, M.Y.H. Wong, P.A. Steck and G.R. Gray, J. Biol. Chem., 254, 5374-5740 (1979). Phase I Study of Intralesional Immunotherapy with Oil-Attached Mycobacterium smegmatis Cell Wall Skeleton and Trehalose Dimycolate, G. Vosika, J. Schmidtke, A. Goldman, R. Parker, E. Ribi and G.R. Gray, Cancer Immunol. Immunother., 7, 221-224 (1980).

170. BCG POTENTIATED ANTITUMOR RESPONSE - ESCAPE MECHANISMS

Hawrylko, E., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

The primary objective of this proposal is to determine the mechanisms by which tumors avoid destruction by the immune response generated in a murine model of BCG potentiated delayed-type hypersensitivity (DTH) and antitumor immunity. Our previous investigations explored how serum factors and suppressor cells from tumor bearing hosts inhibited DTH and antitumor immunity in this system. We recently found that suppressor cells did not affect the augmented DTH response obtained when either BCG primed or unprimed hosts were injected with cyclophosphamide (CY) prior to sensitization. This implies that inhibition of DTH involves the interaction of suppressor cells with CY sensitive cells in the immune host. We now propose to: (1) Identify the phenotype of the CY sensitive cell(s); (2) identify the phenotype of the cells and the conditions required to induce suppressor cells in the tumor bearing host; and (3) determine if serum blocking factors from tumor bearers can inhibit the CY augmented response or whether they also require interaction with CY sensitive cells and, if the latter, then we will identify the phenotype of the interacting cell.

171. THE EFFECT OF BCG CELL WALLS ON TUMOR GROWTH

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The objectives of this research project were to determine if suppressor cells were involved in the immunoregulation of tumor growth. Suppressor cells were directly identified in vitro as cells which caused a depression in blastogenesis responses (Con A and mixed lymphocyte response-MLR) and their effect in vivo was indirectly assessed by what effect they had on tumor growth. We evaluated whether suppressor cells could be identified in tumor bearer animals, in BCGcw immunized animals, and in BCGcw tumor bearer animals. Suppressor cells were identified in all of these situations. The in vivo studies with BCGcw immunized animals showed a consistent enhancement of tumor growth. BCGcw immunization blocked the inductive phase of tumor immunity but it had no effect on established tumor immunity. In vitro studies showed that BCGcw immunization stimulated the development of suppressor cells. The Con A response was 20% of normal whereas the MLR reactivity was less than 10% of normal. Studies to characterize the responsible cell showed that an adherent macrophage cell was the cell which caused the immunosuppression. Future studies will be aimed at modifying the expression of suppressor cells in these various tumor situations. We also plan to determine if these suppressor cells might be used in a positive fashion to modify other immune responses.

172. PHOTOAUGMENTATION AND CARCINOGENESIS

Willis, I., Israili, Z. H., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Dermatology Service, *1670 Clairmont Rd., Decatur, Georgia,* 30033, United States of America

The overall objective of this proposal is to use a highly predictable in vivo animal model, developed in our laboratories, for the rapid induction of squamous cell cancer by UVB light in order to 1) gain better insight into the pathogenesis and treatment of squamous cell cancer, 2) develop a useful in vivo system for more rapid evaluation of cancer chemotherapeutic and immunotherapeutic drugs, and 3) develop more effective sunscreens, drugs and agents.

Studies using BCG immunotherapy revealed striking results in that in 80% of animals treated with 0.5 ml, 1.5 to 2.3 x 10 to the 7th power CFU injected under the base of the tumors, tumors began to regress fairly rapidly. However, in 20% of the animals there was initial partial regression and then subsequent accelerated growth. Since all tumors had been judged as clinically similar prior to initiation of therapy one would expect all tumors to respond similarly. To our amazement tumors that did

not respond to immunotherapy were histopathologically spindle-celled squamous cell carcinomas that closely resembled fibrosarcomas. Using our system of tumor induction we had not previously encountered grade IV type squamous cell carcinomas although the tumors did eventually lead to death in all animals. Current studies are underway to determine a BCG dose schedule and chemotherapy that might be effective in the treatment of these tumors and to determine what led to their development during the course of irradiation.

173. BIOASSAY FOR ANTITUMOR EFFECTS OF R.E. STIMULANTS

Proctor, J. W., Gorecka Tisera, A., Allegheny-Singer Research Corp., *320 E. North Ave., Pittsburgh, Pennsylvania*, 15212, United States of America

OBJECTIVE: To develop a cheap, rapid, comprehensive, and biologically relevant bioassay for screening the anti-tumor activity of biological response modifiers of the reticuloendothelial stimulant class.

APPROACH: To measure the extent of clearance of intravenously injected radiolabelled tumor cells from the lungs following prior administration of BRMs.

PROGRESS: The optimal doses, time for initiation of effects, their duration, and magnitude and, in some cases, optimal routes of administration have been established for C. parvum, BCGs, glucans, pyrans, and MDPs. The results correlate with the subsequent outgrowth of lung tumor nodules, survival, and the increased cell loss from the lung appears to be due to tumor cell death. The method appears to be suitable as a screening method for selecting superior developmental agents and for quality control of existing agents.

174. MECHANISM OF DELAYED HYPERSENSITIVITY AND TUMOR GRAFT REJECTION

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It is the purpose of this project to elucidate the mechanism of the immunologic rejection of tumor grafts and to determine the mechanism of tumor suppression following immunostimulation with BCG. Current areas of investigation are: evaluation of the specificity of immunization evoked by living BCG and fibrosarcoma cells and development of methods for elimination of microscopic metastases remaining after surgery.

BIBLIOGRAPHIC REFERENCES: Zbar, B., Canti, G., Rapp, H.J., Ashley, M.P., Sukumar, S., and Bast, R.C., Jr. Immunoprophylaxis of syngeneic methylcholanthrene-induced murine sarcomas with Bacillus Calmette-Guerin and tumor cells. Cancer Res. 40: 1036-1042, 1980.

175. STUDIES ON THE MECHANISMS OF BCG-INDUCED MODIFICATIONS OF IMMUNOLOGICAL RESPONSES

Sljivic, V. S., Brown, C. A., University of London, St. Mary's Hospital Medical School, Dept. of Immunology, *Norfolk Pl., London W2 1PG, England, United Kingdom*

OBJECTIVE: Mycobacterium bovis (BCG) has been developed as a vaccine for the prevention of tuberculosis and more recently it has been used in experimental and clinical studies on non-specific immunotherapy of malignant disease. Administration of BCG can cause profound changes in the immunological reactivity of the recipient, the details of which are still not fully understood. With respect to humoral responses, these changes can range from marked enhancement to profound suppression depending on a number of factors (Brown et al., Immunol., 38: 481, 1979). The aim of the present project is to investigate the adjuvant and the suppressive effects of BCG by (a) characterizing the cell populations responsible, (b) establishing conditions for the induction of these cells, (c) studying intracellular events associated with the differential effect of BCG, and (d) studying the mechanisms which determine whether administration of BCG results in enhancement or suppression.

APPROACH: Cells prepared from spleens of mice treated with BCG are cultured using the Marbrook culture system in the presence of T-cell-dependent or -independent antigens, and the response to these is evaluated in terms of number of antibody-forming cells. Various cell fractionation procedures are used to deplete or enrich particular populations of cells from untreated and treated animals and these are co-cultured in various combinations. Drugs and metabolic inhibitors are used to characterize further various cell populations and the possible mode of their interaction. Supernatants from cultures of cells from various donors are tested for their ability to modify the response of normal cells. In addition, the effect of administration of BCG on antibody responses in vivo is being investigated.

PROGRESS: This has been reported in the following publications: Brown, Brown and Sljivic: Suppressed or enhanced antibody responses in vitro after BCG treatment of mice: importance of BCG viability, Immunology, 38: 481, 1979; Phagosome/lysosome fusion: a possible prerequisite for the enhancement of antibody responses in vitro by BCG, Mycobacterium leprae and Corynebacterium parvum, Parasite Immunology, 1: 309, 1979; Active suppression masks an underlying enhancement of antibody production in vitro by spleen cells from BCG-treated mice, Immunology, 40: May/June, 1980.

176. MURINE EFFECTOR CELLS

Henney, C. S., Kuribayashi, K., Fred Hutchinson Cancer Research Center, Program in Basic Immunology, *1124 Columbia St., Seattle, Washington*, 98104, United States of America

We seek to characterize the mechanism of action of BCG-induced NK cells and to explore the possibility that this cell type may be linked to the therapeutic usefulness of BCG in causing regression of various neoplasms.

Additionally, we have noted two other activities induced by BCG which may be germane to the regulation of host-tumor interactions. One of these involved a suppressor cell(s) which prevented both the primary induction of cytotoxic T cells and the differentiation of T memory cells in vitro. The other involved a lectin-dependent cytotoxic cell. None of these effector activities were exhibited by cells from non-immunized animals, nor by peritoneal exudates induced with thioglycollate or proteose peptone.

We will characterize these BCG-induced effector cells by studying their rate of appearance, mechanism of induction, specificity of effector function and mode of action. In addition we will use Winn-type assays to evaluate directly the roles of each of these effector cells in the BCG-induced immunotherapy of murine tumors. It is hoped that information from these studies will provide a more rational basis for intervention with BCG in human cancer.

BIBLIOGRAPHIC REFERENCES: Young, W.W., Hakomori, S., Durdik, J.M. and Henney, C.S. Identification of Ganglio-N-Tetraosylceramide As A New Cell Surface Marker For Murine Natural Killer (NK) Cells. J. Immunol. 124:199, 1980. Green, W. and Henney, C.S. The Mechanism of T Cell-Mediated Lysis. C.R.C. Critical Reviews in Immunology, in press, 1980.

177. INDUCTION OF TUMOR RESISTANCE WITH BACILLUS CALMETTE-GUERIN (BCG)-ASSOCIATED TUMOR ANTIGENS

Crum, E. D., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *10701 East Blvd., Cleveland, Ohio*, 44106, United States of America

OBJECTIVES: (1) To examine the immunogenicity of soluble tumor antigens coupled to living BCG; and (2) to examine mechanisms of in vivo resistance to tumor growth to assess the capacity of tumor antigen-BCG complexes to provoke protective immunity.

PLAN: Tumor antigen isolated from methylcholanthrene (Mc)-induced sarcomas and coupled to BCG will be tested for capacity to provoke resistance to tumor growth and examined for the kind of anti-tumor immune response induced in syngeneic Wistar rats.

METHODOLOGY: Tumor antigens will be isolated from tumor extracts by electrofocusing and affinity chromatography, and will be coupled to living BCG by incubation with organisms in aqueous medium. Rats receiving tumor antigen-BCG complexes will be evaluated for tumor resistance by direct tumor challenge. Adoptive transfer of lymphocytes will be used to compare the immune response induced by tumor antigen-BCG complexes with that induced by immunization with irradiated tumor tissue. Lymphocytes able to mediate tumor resist-

ance will be characterized by testing subpopulations of cells isolated by affinity chromatography.

FINDINGS: Thoracic duct lymphocytes from rats immunized with irradiated Mc-induced sarcomas mediate specific tumor resistance in vivo. Mediators appear to be T cells that are in a non-proliferating state when recovered from central lymph, and appear to have a minimum effective life span of two weeks. Their mode of activity is unclear but appears to require mitosis before mediating anti-tumor activity.

CLINICAL RELATIONSHIP: This project examines a possible clinically acceptable mode of inducing host resistance to tumor, and the mechanisms of the immune resistance operative in vivo; both aspects are important in clinical immunotherapy of cancer.

178. EXPERIMENTAL AND CLINICAL IMMUNOLOGY OF UROLOGIC CANCER

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The FANFT-induced bladder tumor in C3H/He mice was carried by subcutaneous inoculation in hind limbs of syngeneic animals. Challenge of previously immunized animals demonstrated the tumor to be immunogenic. Mice received hind limb inoculation of 10 to the 5th power viable tumor cells and were randomized into groups of 20. Treatment was begun either on the day of tumor inoculation (immunoprophylaxis, Group A) or when tumors measured 6 mm in diameter (immunotherapy, Group B). Weekly injections of C. parvum subcutaneously (SC), intraperitoneally (IP) or intralesionally (IL) significantly retarded tumor growth and increased survival in Groups A and B (p less than 0.05 compared to controls). BCG (Tice strain, 5 x 10 to the 4th power viable units) injected into the ipsilateral groin twice weekly retarded tumor growth slightly and increased survival significantly in both Groups A and B. Other routes of administration (SC, IP, IL and contralateral groin IM) and other dosage schedules of BCG were ineffective. Weekly injections of 1 mg of RNA extracted from spleens of tumor-immunized guinea pigs increased survival in Group A (p less than 0.05) but not tumor growth. Higher doses of RNA (3 or 4 mg) not only improved survival but inhibited growth in both Groups A and B. This was the first demonstration of inhibition of a growing, established tumor by immune RNA. Addition of the RNAase inhibitor, sodium dextran sulfate, increased tumor growth inhibition by RNA.

Chemoimmunotherapy experiments showed significant tumor inhibition with Cytoxan, 75 mg/kg, injected IP weekly (p less than 0.01), as well as increased survival (p less than 0.01). Combining Cytoxan with C. parvum IP or SC resulted in significantly greater (p less than 0.05) tumor growth inhibition and survival in both Groups A and B, than using either agent alone. Future experiments will include modification of BCG dosage regimen, treatment with various dosages of RNA to obtain a dose-response relationship, combination of RNA with tumor antigen, combination of RNA with Cytoxan, and exploration of the inhibitory effect of ipsilateral BCG injections.

179. CELL INTERACTIONS INITIATING SYNGENEIC IMMUNITY

Peavy, D. L., Baylor College of Medicine, School of Medicine, Dept. of Microbiology & Immunology, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

Macrophages provide an essential component of the host's immune response to neoplasia. In vivo, macrophages participate as effector cells as evidenced by their presence within tumor foci and their capacity to transfer tumor resistance to syngeneic recipients. Following interactions with lymphocytes and/or soluble products secreted by these cells, macrophages become cytotoxic for tumor cells or, at least, inhibit their growth.

Recent investigations from this laboratory have demonstrated that two powerful immunostimulants, bacterial lipopolysaccharides (LPS) and bacillus Calmette-Guerin (BCG), synergistically inhibit growth of ascites tumors in syngeneic mice. Our preliminary studies suggest that BCG infection activates macrophages extensively while LPS augments the tumoricidal capacity of these cells.

This proposal is designed to investigate the effects of LPS on BCG macrophages in vitro. Using cytochemical and autoradiographic techniques, we will quantitatively determine membrane binding, internalization and intracytoplasmic localization of LPS in macrophages. Other aspects of LPS-macrophage interactions such as phagosome formation, lysosome fusion and extracellular release of lysosomal enzymes will also be explored. Attempts will be made to correlate LPS-induced alterations with increased tumoricidal activity. The effects of drugs which increase or decrease lysosome stability will be analyzed in detail.

By delineating events which characterize LPS interactions at the cellular level, these studies are expected to provide valuable insight and substantial new knowledge regarding the mechanisms of macrophage-mediated cytotoxicity. Furthermore, clinical administration of BCG and LPS, using appropriate therapeutic regimens outlined in this proposal, offers a promising approach for potentiating the host's immunity to autochthonous tumors.

180. EFFECT OF MYCOBACTERIAL VACCINE ON FELINE SARCOMA VIRUS-INDUCED TUMORS IN THE CAT

Cockerell, G. L., Cornell University, Ithaca Campus, New York State College of Veterinary Medicine, Dept. of Pathology, *825 Veterinary Research Tower, Ithaca, New York*, 14853, United States of America

OBJECTIVE: The purpose of this study is to determine the effect of myobacterial (BCG) cell wall preparations on the development and progression of feline sarcoma virus (FeSV)-induced fibrosarcomas in cats, when administered at various times after viral inoculation.

APPROACH: Six- to eight-week-old kittens will be inoculated subcutaneously with FeSV. At 0, 1, 2, 3, and 4 weeks post-infection groups of kittens will either be inoculated (same site as virus inoculation) with a BCG vaccine preparation or the vaccine emulsificant (control). The site of virus inoculation will be palpated and measured daily. All cats will be observed until death or 120 days post-infection, which ever comes first. Complete necropsy examinations will be performed on all cats immediately after death. Comparisons will be made between groups receiving BCG vaccine or emulsificant for time to tumor detection, primary tumor growth rate, development and pattern of metastatic disease, time to death and mortality rate.

181. INDUCTION OF COLON TUMORS IN GUINEA PIGS

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OBJECTIVE: Develop an animal model of large bowel cancer as it occurs in humans; compare immunotherapeutic and surgical approaches to the treatment of large bowel cancer; study methods of early detection of tumors and define the gross microscopic staging of tumors progression or regression before and after treatment.

APPROACH: Animal model: Adenocarcinoma of the colon in the guinea pig will be induced by the intrarectal instillation of the chemical carcinogen methylnitrosourea (MNU). Immunotherapy vs. surgery: Guinea pigs will either receive a direct intratumoral injection of a cell wall preparation of mycobacterium bovis strain BCG or undergo surgical excision of the primary tumor. Controls will receive no tumor treatment. Comparisons between treatments will be made for time to death and behavior of primary and secondary tumors. Early diagnosis, tumor staging: Sequential studies will be be performed during tumor induction and following treatment, including analysis for fecal blood, colonoscopic examination and gross and histological examination of tumor sites.

182. IMMUNOTHERAPY OF CANCER IN PRIMARY AND METASTATIC DISEASE

Wainberg, M. A., Jewish General Hospital, Lady Davis Inst. for Med. Res., *3755 Cote St. Catherine Rd., Montreal, Quebec*, H3T 1E2, Canada

OBJECTIVE: We have demonstrated that apparently successful intra-tumor immunotherapy of a primary lesion frequently leads to a state of minimal residual disease rather than complete eradication of tumor cells, and that progressive metastatic disease can be reactivated following experimental manipulation. These studies were carried out with line 10 hepatocarcinoma cells and strain 2 guinea pigs, which were treated with the

methanol extraction residue (MER) of BCG. We now propose to develop this model of tumor reactivation further, and to attempt the elimination of metastatic growths by a combined immunochemotherapy approach.

APPROACH: Guinea pigs are inoculated intradermally with 10 to the 6th power tumor cells and treated by intranodule injection of BCG or MER one week later. After 4-6 weeks and 2-3 weeks following the disappearance of palpable tumors, the animals are injected both intra-venously and at the site of initial tumor implantation with 3M KCl solubilized extracts of tumor cells. Injection of BCG into the regional lymph nodes coupled with systemic chemotherapy is then attempted beginning 3 weeks later in an effort to prevent enlargement of palpable metastases which have by that time often started to grow.

183. CANINE TUMOR IMMUNOLOGY - FREDERICK COOPERATIVE STUDY OF NEOPLASMS

Shifrine, M., University of California, Davis Campus, Lab. for Energy Related Health Research, *Davis, California*, 95616, United States of America

Aging female beagles on a life-span study of the effects of radionuclides have mammary tumors similar in morphology and behavior to those in women. Treatment by local injection of BCG is effective in guinea pigs in erradicating metastasis to draining lymph nodes. We propose to treat mammary tumor cases from our pilot study to determine the efficacy of BCG treatment in the dog as a model for humans. Cobalt-60 high-risk dogs will be typed for histocompatibility antigens. This will provide information on the relation of dog leukocyte antigen (DLA) types and radiation resistance and leukemogenesis, permit bone marrow (or other cells) transplantation into matched recipients, and provide data on such therapeutic approaches and data on mechanisms of leukemic recurrence in transplantation recipients.

184. STUDY OF CANCER IN DOGS

Theilen, G. H., University of Califc nia, Davis Campus, Agricultural Experiment Station, Depts. of Surgery/Medicine, *Davis, California*, 95616, United States of America

OBJECTIVE: Determine if increasing the immunologic capabilities of the affected animals will assist in tumor regression.

APPROACH: Dogs with spontaneous neoplasms will be injected with bacillus Calmette-Guerin to determine if the hyperreactivity caused by the adjuvant effect will result in regression of tumors. The types of tumors will be determined by pathologic evaluation before animals are placed on treatment.

185. ACTIVE IMMUNOTHERAPY AND IMMUNE MEDIATED REGRESSION OF BOVINE OCULAR CARCINOMA

Pierce, K. R., Joyce, J. R., Pack, F. D., Texas A & M University, College Station Campus, School of Veterinary Medicine, Dept. of Veterinary Pathology, *College Station, Texas*, 77843, United States of America

OBJECTIVE: Prepare and use immunotherapeutic reagents in cattle with ocular carcinoma. Ascertain the value of urinary polyamine levels as an indicator of effectiveness of cancer therapy in cattle. Establish and use a xenograft system to maintain bovine ocular carcinoma in nude mice and study immunologic augmentation by immunotherapy in the mice.

APPROACH: A phenolic extract of bovine ocular carcinoma tissue (specific immunostimulant) and extracts or cell walls of Mycobacterium bovis, strain BCG, will be prepared by established methods and adnministered to cattle with ocular carcinoma. An assay for urinary polyamines will be adopted and used in cattle with ocular carcinoma. Nude mice, with and without whole body irradiation, will be implanted subcutaneously with ocular carcinoma tissue used to prepare specific immunostimulant (20 mice per cow).

186. IMMUNOPROPHYLAXIS OF 'CANCER EYE' IN CATTLE

Kleinschuster, S. J., Utah Higher Education System, Utah State University, School of Agriculture, Dept. of Animal Dairy & Veterinary Sciences, *Agricultural Science Bldg., Room 223A, Logan, Utah*, 84321, United States of America

OBJECTIVES: Study the effect of a vaccine consisting of BCG cell wall in water/mineral oil emulsion on the development of malignant bovine ocular squamous cell carcinoma from benign precursor lesions. Utilizing four experimental animal groups (total 240 cattle), evaluate the prophylactic benefit provided by the vaccine. These groups include animals receiving: active BCG cell wall vaccine; inactive, sham BCG vaccine; no treatment; and surgical removal of the benign lesion. At monthly intervals, monitor each animal for immunologic status by skin testing with DNCB and PPD. Collect and cryopreserve for later examination at the discretion of the Project Officer: tumor tissues; lymphocytes; peritoneal cells; serum; and red cells.

187. IMMUNOTHERAPY OF PRIMARY AUTOCHTHONOUS CANCER

Rapp, H. J., Hunter, J. T., Sukumar, S., Ashley, M. P., Yarkoni, E., Zbar, B., Kleinschuster, S. J., Rice, J., Baker, A. R., Rosenberg, S. A., Bier, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cellular Immunity Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Mammals with naturally occurring or experimentally induced primary autochthonous cancer are being studied as guides to the immunotherapy of human cancer. It is hoped that these guides will provide information more relevant to the treatment of human cancer than that derived from studies with transplanted tumors. Cattle with ocular squamous cell carcinoma ('cancer eye') have been treated successfully by the injection of BCG cell wall vaccine directly into the tumor. Primary autochthonous tumors of mice, rats, dogs and humans are also being studied.

2. C. PARVUM

188. COMPARATIVE TESTING OF VACCINES FROM C. PARVUM

Cummins, C. S., Virginia Polytechnic Inst. & State University, School of Agricultural & Life Sciences, Dept. of Microbiology, *Blacksburg, Virginia*, 24061, United States of America

Investigations will be continued into the conditions of production and the chemical nature of the reticulo-stimulatory substance in vaccines from anaerobic coryneforms (C. parvum vaccines). A continuing search will be made for other bacteria which have similar activity.

189. BIOASSAY FOR ANTITUMOR EFFECTS OF R.E. STIMULANTS

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The summary for this project is under entry no. 173.

190. EXPERIMENTAL AND CLINICAL IMMUNOLOGY OF UROLOGIC CANCER

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The summary for this project is under entry no. 178.

191. IMMUNOLOGICAL ACTIVITY OF EUKARYOTIC AND PROKARYOTIC CELLULAR COMPONENTS

Cantrell, J. L., Ribi, E. E., McLaughlin, C. A., Schwartzman, S. M., Springer, G. F., Wheat, R., Sasaki, J., Tuttle, R. T., Zighelboim, J., Murahata, R. I., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Allergy & Infectious Diseases, Molecular Biology Section, *Hamilton, Montana*, 59840, United States of America

The main goal of this project is the elucidation of the immunological response induced by isolated microbial components and tumor-associated antigens that are efficacious antitumor agents. In the past year, attempts were made to: 1. Evaluate fractions of Corynebacterium parvum prepared by phenol-water extraction for lymphoreticular stimulation and antitumor activity, 2. isolate, in soluble form, the component from C. parvum responsible for tumor regression, 3. evaluate inhibition of immunity and induction of suppressor cells with components isolated from microorganisms, 4. evaluate tumor-associated antigens isolated from murine tumor cells and guinea pig ascites fluid for antitumor activity, and 5. determine the cross-reactivity between tumor cells and microbial components.

BIBLIOGRAPHIC REFERENCES: Cantrell, J. L., McLaughlin, C. A., and Ribi, E.: Efficacy of tumor cell extracts in immunotherapy of murine EL-4 leukemia. Cancer Res. 39: 1159-1167, 1979.

192. IMMUNOLOGICAL BASIS OF TUMOR REGRESSION

North, R. J., Trudeau Inst. Inc., *P.O. Box 59, Saranac Lake, New York*, 12983, United States of America

Experiments will be performed to determine the mechanistic basis of endotoxin-induced tumor regression. Additional syngeneic tumors will be examined to test the hypothesis that the only tumors that completely regress in response to parenteral injection of endotoxin are those that are immunogenic enough to evoke the generation of a state of concomitant antitumor immunity. Concomitant immunity will be investigated in terms of the properties of the sensitized T-cells that mediate it, and in terms of whether the antitumor action of endotoxin is based on its capacity to potentiate the production of tumor-sensitized T-cells. The substitution of an intratumor delayed-sensitivity response to C. parvum or tumor specific immunity, in making tumors susceptible to endotoxin therapy, will be studied in terms of the nonspecific effector mechanism that C. parvum causes to develop in the tumors.

BIBLIOGRAPHIC REFERENCES: Berendt, M.J., North, R.J., and Kirstein, D.P. 1978. The immunological basis of endotoxin-induced tumor regression: Requirement for T-cell-mediated immunity. J. Exp. Med. 148: 1550. Berendt, M.J., North, R.J., and Kirstein, D.P. 1978. The immunologic basis of endotoxin-induced regression: Requirement for a pre-existing state of concomitant antitumor immunity. J. Exp. Med. 148: 1560.

193. SELECTIVE MODULATION OF CELL-MEDIATED CYTOTOXIC REACTIONS - AN APPROACH TO STUDY THEIR ROLE IN TUMOR REJECTION (IN VIVO)

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SPECIFIC AIMS: 1. To investigate modulation of natural killer (NK) cell activity by Corynebacterium parvum and its derived fractions. 2. To investigate the effects of C. parvum derived fractions on primary and memory cell-mediated immunity to a syngeneic tumor inoculum. 3. To establish the contributions of NK cell activity and specific primary and memory cell-mediated cytotoxicity to tumor rejection in vivo. 4. To investigate pharmacologic means of augmenting C. parvum's beneficial effects on tumor rejection.

194. EFFECT OF C. PARVUM ON ACTIVATION OF MACROPHAGES AND ITS POSSIBLE ROLE IN DIMETHYL HYDRAZINE INDUCED COLONIC CANCER IN RATS

Steer, H. W., John Radcliffe Hospital, Nuffield Department of Surgery, *Headington, Oxford, England, United Kingdom*

OBJECTIVE: To determine what effect activation of the immune system has on the effluent cells from the gastrointestinal tract and to examine what effect such activation has on colonic cancer.

APPROACH: Corynebacterium parvum is known to activate the immune system particularly as far as macrophages are concerned. Such activation has been shown to have a beneficial effect on certain tumors, for example, mammary carcinoma. It is proposed to use the rat model (MacPherson and Steer 1979) to study the effect of C. parvum on this gut effluent. It is planned to induce colonic cancers in a group of rats by using dimethyl hydrazine. The gut effluent will be examine in these tumor-bearing animals before and after activation of the immune system with C. parvum.

BIBLIOGRAPHIC REFERENCES: MacPherson, G.G. and H.W. Steer (1979). Properties of mononuclear phagocytes derived from the small intestinal wall of rats. In function and structure of the Immune System ed. Mueller-Ruchholtz and Muller-Hermelink. pp. 433-438.

195. THE ROLE OF MACROPHAGE SUBCLASSES IN TUMOR IMMUNITY

Fishman, M., Veit, B. C., Morrison, M., St. Jude Childrens Research Hospital, Division of Immunology, *332 N. Lauderdale St., P.O. Box 318, Memphis, Tennessee*, 38101, United States of America

The role of the macrophage effector cells in tumor immunity is to be studied in light of the functional heterogeneity of these cells. The cytotoxic activity (tumor growth inhibition and tumor lysis) of the light density population of macrophages from normal or stimulated animals will be compared to the activities of activated macrophages and subpopulations of activated macrophages obtained from animals injected with C. parvum. These comparisons will include selective recognition for neoplastic cells and enzymatic activities associated with macrophage activation. The mode of action of the stimulated macrophage subpopulations that inhibit or augment tumor growth will be elucidated in respect to interaction kinetics, effects on tumor cell membranes and tumor cell metabolism. In addition, the in vitro tumor-growth inhibitory activity of macrophage subpopulations will be assessed in vivo.

BIBLIOGRAPHIC REFERENCES: Weinberg, D.S., Fishman, M., and Veit, B.C.: Functional heterogeneity among peritoneal macrophages. I. Effector cell activity of macrophages against syngeneic and xenogeneic tumor cells. Cell. Immunol. 38:94-104, 1978. Fishman, M., and Weinberg, D.S.: Functional heterogeneity among peritoneal macrophages. II. Enzyme content of macrophage subpopulations. Cell. Immunol. 45:437-445, 1979.

196. THERAPY EFFECTS ON TUMOR KINETICS AND IMMUNE PARAMETERS

Fisher, B., Gunduz, N., Wolmark, N., Ratajczak, H., University of Pittsburgh, School of Medicine, Dept. of Surgery, *3550 Terrace St., Pittsburgh, Pennsylvania*, 15261, United States of America

Investigations carried out in this laboratory have been directed toward evaluating the use of combined modality treatments, i.e., immunochemotherapy, on the growth of a primary tumor and metastases. It was observed that when C. parvum (CP) was administered i.p. asynchronously in combination with cyclophosphamide (CY) at weekly intervals to C3HeB mice bearing an established growing C3H mammary adenocarcinoma, tumor growth inhibition was achieved which was greater than that resulting from either agent alone. In additional investigations, other chemotherapeutic and non-specific stimulating agents were substituted for or added to the CY, CP combination. None, when substituted, produced results equal to that combination, nor did their addition improve the results. We have attempted to identify host responses resulting from the therapy which might be responsible for the overall effectiveness of the combination. None of the investigations provided clear evidence implicating any of the host factors in the general success of treatment. Of particular interest has been the observation that in all experiments employing this combination of therapy, the effect on tumor growth varied from mouse to mouse despite the fact that tumors in any one study were from the same inoculum and were in syngeneic hosts. Whether this divergence was the result of disparate host modulation or differences in tumor characteristics was speculative. The present investigations with two C3H mammary tumors in the same host indicate that the tumors rather than the host are responsible for the observed variation in treatment effectiveness. They suggest that each inoculum contains a heterogeneous tumor cell population so that aliquots of the suspension upon transfer produce, by chance, clones giving rise to tumors having variable susceptability to the therapy. Preliminary investigations evaluating tumor cell kinetics have shown that the cytokinetics of those tumors responding best to treatment differ from those responding least. Investigations during the coming year will continue to assess tumor and host factors in responders and non-responders to chemo-immunotherapy.

197. ANTITUMOR ACTIVITY OF BCG, C. PARVUM AND GLUCAN

Megirian, R., Loose, L. D., Union University, Albany Medical College, School of Medicine, Dept. of Pharmacol. & Exptl.

Therapeutics, *47 New Scotland Ave., Albany, New York,* 12208, United States of America

The summary for this project is under entry no. 168.

198. ENZYME AND TUMOR NECROSIS FACTOR LEVELS IN TISSUES OF C. PARVUM TREATED RODENTS

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This is part of a broader project. A summary of this subproject is not available.

3. OTHER MICROBIAL ADJUVANTS

199. SURFACE CHEMISTRY OF MYCOBACTERIA

Goren, M. B., Liav, A., University of Colorado, Health Sciences Center, National Jewish Hospital & Research Center, Molecular & Cellular Biology Division, *3800 E. Colfax Ave., Denver, Colorado,* 80206, United States of America

Our research deals principally with lipids of virulent tubercle bacilli. Cord factor and the mycobacterial sulfatides are two classes of trehalose-containing glycolipids from M. tuberculosis which are implicated in virulence and in pathogenesis of the disease. Both substances also have antitumor activity in the line 10-strain-2 guinea pig system; but only cord factor (and closely related analogs) is active against certain murine fibrosarcomas. Our studies involve synthesis of pseudocord factors based upon 'trehalose dicarboxylic acid' and 6,6-dideoxytrehalose to find inexpensive substitutes for natural cord factors, potentially useful in tumor immunotherapy and to determine the influence of defined structural changes on biological activities.

The sulfatides, and other polyanionic agents, have been implicated in inducing phagosome-lysosome fusion dysfunction in cultured macrophages. Thus the mycobacterial sulfolipids are probably functional in promoting the intracellular survival of M. tuberculosis. Our current studies seek to define whether macrophage stimulation/activation abrogates the block to fusion; and the influence of this block on bactericidal activity.

BIBLIOGRAPHIC REFERENCES: Goren, M.B. and K.S. Jiang. Alpha-D-(glucopyranosyluronic acid) alpha-D-(glucopyranosiduronic acid) and simple derivatives. Carbohydrate Res. 79, 225-234 (1980). Liav, A. and M.B. Goren. Synthesis of 6,6'-diacylated trehaloses via hexa-O-benzyl trehalose. Carbohydrate Res. 81, C1-C3 (1980).

200. STRUCTURE AND BIOLOGICAL ACTIVITY OF ENDOTOXINS

Von Eschen, K. B., Ribi, E. E., McLaughlin, C. A., Cantrell, J. L., Parker, R. A., Strain, S. M., Rudbach, J. A., Milner, E. C., Williams, J. C., Sasaki, J., Nakamura, M. J., Wells, S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Allergy & Infectious Diseases, Laboratory of Microbial Structure & Function, *Hamilton, Montana,* 59840, United States of America

The main objective of this project is to determine the minimal chemical and structural properties of bacterial endotoxins necessary to elicit several biological and immunologic responses. The purpose is to identify, by fractionation of different endotoxins and chemical, physical and biological analyses of the individual fractions, the component(s) of the macromolecular complex responsible for the antitumor effects of some endotoxins. Techniques are being developed to assess the cellular nature of the immunomodulatory activities of different endotoxin materials. Also, this project continues to perform standard bioassays of endotoxic materials contributed by several members of the LMSF.

201. IMMUNOTHERAPY WITH DEFINED BACTERIAL COMPONENTS

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Components of mycobacteria injected in oil/water emulsions into syngeneic tumors growing in the skin of guinea pigs of mice caused the tumors to regress. Animals cured of their tumors were resistant to rechallenge with the same tumor. A mixture of living tumor cells and emulsified, heat-killed BCG cells injected intradermally into guinea pigs with growing dermal tumor and microscopic lymph node metastasis caused eradication of the dermal tumor and lymph node metastases.

BIBLIOGRAPHIC REFERENCES: Yarkoni, E. and Rapp, H.J.: Influence of surfactant concentration on the antitumor activity of emulsified components of mycobacteria. Cancer Res. 40: 975-978, 1980. Yarkoni, E. and Rapp, H.J.: Influence of type of oil and surfactant concentration on the efficacy of emulsified Mycobacterium bovis (BCG) cell walls to induce tumor regression in guinea pigs. Infect. Immun. 28: 881-886, 1980.

202. MICROBIAL COMPONENTS IN CANCER IMMUNOTHERAPY

Ribi, E. E., McLaughlin, C. A., Cantrell, J. L., Von Eschen, K. B., Zbar, B., Brehmer, W., Richman, S., Granatek, C. H., Schwartzman, S. M., Vosika, G., Parker, R., Minden, P., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Allergy & Infectious Diseases, Laboratory of Microbial Structure & Function, *Hamilton, Montana,* 59840, United States of America

The major objectives of this project continue to be to identify nonviable, chemically defined microbial components that are efficacious antitumor agents and develop and supply such preparations in stable, standardized form for both experimental and clinically applied immunotherapy. The intent of the research is to circumvent the use of infectious agents and, by preparing synthetic analogues, provide alternative substances that are antagonistic or that do not produce undesirable biological responses.

BIBLIOGRAPHIC REFERENCES: Zbar, B., Canti, G., Ashley, M. P., Rapp, H. J., Hunter, J. T., and Ribi, E.: Eradication by immunization with mycobacterial vaccines and tumor cells of microscopic metastases remaining after surgery. Cancer Res. 39: 1597-1603, 1979. Brehmer, W., Huckauf, H., and Ribi, E.: Immuntherapie mit BCG sowie mykobakteriellen frakitonen und versuche zur anwendung beim bronchial-karzinom. Praxis Pneumonologie 33: 358-365, 1979.

203. EXTRACORPOREAL IMMUNOADSORBENTS IN IMMUNOTHERAPY

Terman, D. S., Solomon, B., Pitt, A., Colton, C., Bier, M., Bridges, C. J., Baylor College of Medicine, School of Medicine, Dept. of Medicine, *1200 Moursund Ave., Houston, Texas,* 77025, United States of America

In previous studies in this laboratory, several extracorporeal immunoadsorbents have been developed consisting of immobilized antigens, antibodies and enzymes which have shown a capacity to extract their respective antigens, antibodies and substrates from circulating plasma in vivo. With these systems, disease processes including experimental nephrotoxic glomerulonephritis and hyperacute renal xenograft rejection have been arrested or attenuated. More recently, the application of this approach to systemic lupus erythematosus in man resulted in substantial renal functional and morphologic improvement. We have now extended this approach to therapy of spontaneous breast adenocarcinoma in the dog. Employing formalin stabilized staphyloccocus aureus Cowan I (SAC) embedded in a membrane filtration system we have observed substantial tumoricidal effects in spontaneous canine breast adenocarcinoma. The tumoricidal response is rapid and specific for primary and multiple metastatic sites with minimal toxicity to the host. In some instances, healing of large ulcerated tumor areas has been observed. Serologic and immunohistochemical evaluation of tissue suggests that the tumoricidal response is initiated by tumor specific antibodies which are generated by the extracorporeal perfusion procedure. The proposed studies would extend this approach from dogs to clinical trials in breast adenocarcinoma in humans to include serologic, immunologic and immunohistochemical assessment. Concomitantly, studies will continue in the canine model employing extracorporeal perfusion over purified protein A, aggregated IgG columns and forced flow electrophoresis. Promising results have shown tumoricidal effects associated with these systems which may be markedly augmented by the concomitant use of carefully timed cytotoxic drug therapy in the post-perfusion period. Projected studies will continue the assessment of these systems and their interaction with existing modes of chemotherapy and immunotherapy. Improvements in extracorporeal cell-separation technology and refinement in immobilization techniques will be tested

in vivo in our canine models of SLE and breast cancer. Extracorporeal immunoadsorption may thus represent a major new therapeutic instrument for clinicians which at the same time may enhance our understanding of the pathophysiology of numerous neoplastic and autoimmune diseases.

BIBLIOGRAPHIC REFERENCES: Terman, D.S., Garcia-Rinaldi, R., McCalmon, R., Modification of Hyperacute Renal Xenograft Rejection after Extracorporeal Immunoadsorption of Heterospecific Antibody. Int. J. Artif. Organs 2, 35-41, 1979. Terman, D.S., Moore, D., Collins, J. Detection of Immune Complexes in Sera of Dogs with Rheumatic and Neoplastic Diseases by 125I C1q Binding Test. J. Comparative Pathology 89, 1-7, 1979.

204. NEW APPROACHES TO IMMUNOTHERAPY

Scibienski, R. J., University of California, Davis Campus, School of Medicine, Dept. of Medical Microbiology, *Davis, California,* 95616, United States of America

Previous experiments carried out in this laboratory indicate that the adjuvant properties of bacterial lipopolysaccharide can be specifically enhanced by irreversibly coupling protein antigen to it. We have further shown that the immune response to a protein antigen coupled to LPS is totally dependent on T cell function and have demonstrated that T lymphocytes are in fact specifically stimulated by these complexes. Other studies have indicated that certain modifications of either the antigen or the LPS will result in preparations which stimulate T lymphocytes without concomitantly inducing antibody synthesis. We thus have a method by which one can induce only CMI or both CMI and antibody synthesis. The purpose of the present proposed research is to determine whether this approach is feasible for immunotherapy of cancer. It is our intent to extract tumor antigens, couple them to LPS or mycobacterial cells, and then assess the ability of the tumor antigen-adjuvant complexes to induce immunoprophylaxis to transplanted tumor. Those complexes which are found to be active by this assay will then be assessed for immunotherapeutic effect on ongoing tumors. In both the immunoprophylaxis and immunotherapeutic studies we will correlate any observed effects with various in vitro measures of immune parameters.

205. MECHANISMS OF IMMUNOPOTENTIATION BY COMPONENTS OF MICROBES

McLaughlin, C. A., Ribi, E. E., Cantrell, J. L., Von Eschen, K. B., Schwartzman, S. M., Toubiana, R., Goldberg, E. P., Granatek, C. H., Jones, G., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Allergy & Infectious Diseases, Laboratory of Microbial Structure & Function, *Hamilton, Montana,* 59840, United States of America

It is the purpose of this project to delineate, at the cellular and molecular levels, the mechanisms of action of microbial components in immunopotentiation. In the past year, two major efforts have been made: 1. Elucidating the nature of biological response to treatment of tumors with synthetic analogs of mycobacterial cell wall components combined with endotoxic extracts obtained from Salmonella typhimurium Re mutants, and 2. establishing the structural features of analogs of adjuvant dipeptides required for antitumor activity when combined with trehalose dimycolate.

BIBLIOGRAPHIC REFERENCES: McLaughlin, C. A., Ribi, E. E., Goren, M. B., and Toubiana, R.: Tumor regression induced by defined microbial components in an oil-in-water emulsion is mediated through their binding to oil droplets. Cancer Immunol. Immunother. 4: 109-113, 1978. McLaughlin, C. A., Hargrave, S. L., Bickel, W. D., and Ribi, E.: Synergistic activity of components of mycobacteria and mutant Salmonella in causing regression of line-10 tumors in guinea pigs. Cancer Res. 39: 1766-1771, 1979.

206. MODE OF ACTION OF A STIMULATOR OF TUMOR RESISTANCE

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Work in our laboratory has focused on the nature of the immunomodulatory effects of bacterial lipopolysaccharide. We shall continue to investigate the role of regulatory T cells and accessory macrophages in LPS stimulatory and inhibitory effects in the antibody response in vivo and in vitro, utilizing syngeneic LPS responder and non-responder strains of mice. In addition, we are examining the ability of lipid-A associated protein and muramyl dipeptide, both bacterial cell wall constituents with adjuvant properties, to exert suppressive and stimulatory effects on the antibody response in LPS non-responder strains of mice. Finally, we shall characterize and determine the cellular requirements for the synergy we have observed between LPS and T cell replacing factors in CBA/N mice, a strain with B cell maturational defects and the absence of a B cell surface antigen which is involved in B cell triggering.

BIBLIOGRAPHIC REFERENCES: Uchiyama, T. and Jacobs, D.M. Modulation of immune response by bacterial lipopolysaccharide (LPS). Cellular basis of stimulatory and inhibitory effects of LPS on the in vitro IgM antibody response to a T-dependent antigen. J. Immunol. 121:2347-2351, 1978. Uchiyama, T. and Jacobs, D.M. Modulation of immune response by bacterial lipopolysaccharide (LPS). Multifocal effects of LPS-induced suppression of the primary antibody response to a T-dependent antigen. J. Immunol. 121:2340-2346, 1978.

207. BIOLOGICAL ACTIVITIES OF B. PERTUSSIS

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The purpose of the proposed research is to identify the mechanism by which the lymphocytosis-promoting factor (LPF) from B. pertussis exerts its effect, not only in causing lymphocytosis but also with respect to its other activities as histamine-sensitizing factor and as a potent mitogen for murine T-cells, which also results in the generation of non-specific cytotoxic lymphocytes. In addition, the activity of LPF as a mitogen for human lymphocytes will be explored. The relationship between LPF and other products of B. pertussis responsible for 1) adjuvanticity for conventional antibody formation as well as for homocytotropic antibody production and 2) protection against clinical pertussis will be explored. B. pertussis also enhances delayed hypersensitivity, and experiments on the potential use of B. pertussis for immunotherapy of tumors are being carried out using mouse melanoma as the model system.

BIBLIOGRAPHIC REFERENCES: Morse, S.I., Lymphocytosis-promoting factor of Bordetella pertussis: Isolation, Characterization, and Biological Activity., J. Inf. Dis., 136,S234-S238, 1977.

208. IMMUNOSTIMULATORY AND ANTINEOPLASTIC EFFECTS OF PROPIONIBACTERIUM GRANULOSUM KP-40 (CORYNEBACTERIUM ACNES) CELL WALL FRACTIONS

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Corynebacterium parvum (CP) was for a long time used in immunotherapy of experimental and clinical neoplasms, although various side effects were observed and varying antineoplastic effectiveness noted in clinical trials. In our former experiments the most active strain of Propionibacterium (formerly known as Corynebacterium) was selected from about 300 strains and identified as Propionibacterium granulosum strain KP-40 (P.g.). Immunostimulatory and antineoplastic activities were identified in cell walls, and active fractions from cell walls were isolated.

The following main subjects will be explored in the project: isolation and chemical characterizaton of cell wall fractions possessing immunostimulatory and antineoplastic activities; cytostatic effect of P.g. and isolated fractions on cancer cells (evaluation of cell cycle and its phases); post-irradiation recovery of hemopoietic and immune systems after treatment with P.g.; antineoplastic effect of P.g. after intraperitoneal and intratumoral injection; clinical trials with use of P.g. and isolated cell wall fractions in advanced lung tumors (in combination with cell-synchronizing chemotherapy) and in gastrointestinal adenocarcinoma (intratumoral injections through an endoscope).

209. STIMULATORY EFFECT OF IMMUNIZATION ON TUMORIGENESIS

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Immunization with small amounts of inactivated Moloney leukemia virus can increase tumorigenesis by Moloney sarcoma virus. Immunization is a potential mode of immunotherapy and of prevention of cancer; therefore, it is necessary to determine whether increased tumorigenesis after immunization is a general phenomenon. The purpose of this study is twofold: (1) To determine whether increased tumorigenesis after immunization is a general phenomenon in virus-induced primary neoplasia and (2) to define some of the immunologic parameters of the stimulation, particularly its immunologic specificity and its mediator(s). The information obtained from this study will be used to evaluate whether stimulation is a potential hazard of immunization and, further, it will aid in the analysis of the mechanism of stimulation. More knowledge about this mechanism will help in deciding whether or not the stimulatory effect of immunization can be avoided.

210. ANTITUMOR ACTIVITY OF GLYCOLIPID-ENDOTOXIN MIX

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A mixture of mycolylacetyltrehalose from Mycobacterium tuberculosis and endotoxin preparation from the Re mutant of Salmonella typhimurium in oil droplets has a potent antitumor activity when tested against transplanted hepatomas in syngeneic guinea pigs. The endotoxin preparation has now been shown to contain trace amounts of an unknown component that is required along with the purified endotoxin for the tumor regression activity. Results suggest that this factor could be a peptide-containing lipid. Purification and characterization of this factor is in progress.

We have now developed a suitable murine tumor assay system to test immunotherapeutic agents. CDF1 mice and P-388 tumor cells are used in this assay. We are now studying the effects of the glycolipid-endotoxin mixture on this assay system.

BIBLIOGRAPHIC REFERENCES: Takayama, K., N. Qureshi, H.C. Jordi, and H.K. Schnoes. Separation of homologous series of mycolic acids from Mycobacterium tuberculosis H37Ra by high performance liquid chromatography. In Biological/Biomedical Applications of Liquid Chromatography. Hawk, G.L. (ed.). Marcel Dekker, Inc., New York, 1978. Qureshi, N., K. Takayama, H.C. Jordi, and H.K. Schnoes. Characterization of the purified components of a new homologous series of alpha-mycolic acids from Mycobacterium tuberculosis H37Ra. J. Biol. Chem. 1978 (in press).

211. IMMUNOPREVENTION OF SPONTANEOUS TUMORS WITH VIRAL AND CELLULAR VACCINES

Kelloff, G. J., Kende, M., Stephenson, J. R., Dinowitz, M., Veronese, F., Witz, I., Nicolin, A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Viral Immunology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

This project has the long-range goal of prevention by immunological means of naturally occurring or chemically induced tumors in animal model systems that have viral, immunologic and epidemiologic similarities to human tumors. To this end, the project has defined the spontaneous tumor and endogenous type C virus expressions in colonies of untreated aging mice and has measured the host's natural and induced immune response to the same. Certain immunization regimens have been found by this project and other investigators to induce an immune response in the host that is effective in preventing experimentally induced tumors and type C virus expressions. Therefore, the major emphasis of the project now is to specifically define the effector immune responses that occur and are responsible for the successful prevention of tumors, whether naturally occurring or induced. To achieve this, additional techniques specifically involving lymphocyte separation and characterization are being established. An equivalent emphasis is being placed on the nature and specificity of the target antigens that these immune responses are directed against, especially with regard to the gene products of the type C virus including the more recently defined transforming proteins.

BIBLIOGRAPHIC REFERENCES: Kende, M., Hill, R., Dinowitz, M., Stephenson, J.R., and Kelloff, G.J.: Naturally occurring lymphocyte mediated immunity to endogenous type C virus in the mouse. Blocking of the lymphocyte reactivity with antisera to the virus. J. Exp. Med. 149: 358-371, 1979.

212. MIXED BACTERIAL TOXINS AS IMMUNOPOTENTIATORS IN CANCER THERAPY (DOGS, CATS)

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This is part of a broader project. A summary of this subproject is not available.

4. OTHER ADJUVANTS

213. IMMUNOTHERAPY OF CANCER WITH A (1-3)-BETA-D-GLUCAN AND ITS DERIVATIVES

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OBJECTIVE: To develop excellent immunoadjuvants for cancer immunotherapy or immunochemotherapy.

APPROACH: For the primary assay, subcutaneous solid tumor of Sarcoma 180 in ICR mice is used. A comparison is made between the weight of the tumors in the treated and the control mice on day 35. In a progressing study, other tumor-host systems will be used.

(1-3)-Beta-D-Glucan (TAK-N) was prepared from the culture broth of Alcaligenes faecalis var. myxogenes, IFO 13140. Some derivatives of TAK-N have been developed to be tested.

PROGRESS: The carboxymethyl derivative (CM-TAK) which has an average degree of substitution (DS) of around 0.5 showed a stronger antitumor activity than the original TAK-N. When DS exceeded 0.7, the activity dropped sharply. The optimum effective dose in one-shot injection 7 days after tumor implantation showed a wider range than TAK-N. The antitumor spectrum, however, was wider in the original TAK-N than in CM-TAK. Pyranyl-, furanyl-, carbamoyl-, monomethylcarbamoyl-, and dimethylcarbamoyl-derivatives were made. In a preliminary test, the dimethylcarbamoyl-TAK showed a very strong antitumor activity and a wide antitumor spectrum.

214. DEVELOPMENTAL THERAPEUTICS - GLUCAN

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Our previous studies have demonstrated that glucan isolated from Saccharomyces cerevisiae is a unique reticuloendothelial (RE) agent and induces a profound enhancement in humoral and cell-mediated immunity. Relative to the anti-tumor effect of glucan, glucan administration is associated with inhibition of tumor growth, and regression as well as an enhancement in survival of mice and rats when challenged with various tumor models. Since we have previously reported that specific water soluble di- and oligosaccharides of glucan have RE stimulating activity, efforts will be made to develop water soluble tumor inhibitory fractions of yeast glucan. The proposed research provides for 1) the continued development of glucan as an immunostimulant, 2) the assessment of the mechanism of action of particulate and soluble glucan, 3) the interrelationships between glucan immunotherapy and chemotherapy, and 4) the assessment of the ability of glucan to modify tumor induced anergy as well as bacterial, viral and fungal infections in control and tumor-bearing mice. It is apparent from the composite experimental and clinical data obtained to date that water soluble glucan derivatives offer significant potential in immunotherapy as well as immunoprophylaxis.

BIBLIOGRAPHIC REFERENCES: Serum Lysozyme: an index of macrophage function. P.L. Kokoshis and N.R. Di Luzio. J. Reticuloendothel. Soc. 25, 85-99, 1979. The role of Kupffer cells in homeostasis. N.R. Di Luzio. In: Problems in Liver Disease. D.S. Davidson, ed., Stratton Intercontinental Medical Book Corp., New York, p. 113, 1979.

215. MOUSE TUMOR ASSAYS FOR LYMPHOKINE IMMUNOTHERAPY

Papermaster, B. W., Gilliland, C. D., Cancer Research Center, *Garth Ave. & Hwy. 40, P.O. Box 1268, Columbia, Missouri*, 65201, United States of America

In the past year an immunotherapeutic model designed to utilize lymphokine preparations for the treatment of systemic, disseminated L1210 lymphoma was being developed. The purpose of these studies has been to develop lymphokine treatment as an adjunct to chemotherapy. Collaborative studies with the Southern Research Institute of Birmingham, Alabama - Dr. Frank Schabel's laboratory - have helped us to develop a quantitative approach to this study. Graded doses of cell inoculum and drug are utilized with various regimens of lymphokine treatment. The system was developed by using the same strain of mice, DBA/2 (Jackson Labs, Bar Harbor, Maine), in both laboratories, and tumors were obtained from the DCT branch of the National Cancer Institute.

BIBLIOGRAPHIC REFERENCES: Papermaster, B.W., Gilliland, C.D., McEntire, J.E., Smith, M.E., and Buchok, S.: Lymphokine-mediated immunotherapy studies. Cancer 45:1247-1253, 1980. Papermaster, B.W., McEntire, J.E., Gilliland, C.D., Buchok, S. and Smith, M.E.: Characterization of lymphotoxin and tumor inhibition properties in a lymphokine preparation derived from the cultured mouse L1210 lymphoma. In Biochemical Characterization of Lymphokines, de Weck, A.L., Kristensen, F. and Landy, M. (Eds.). Academic Press, New York, 1980, pp. 193-198.

216. PRODUCTION AND ACTION OF TUMOR NECROSIS FACTOR (AS A BASE FOR IMMUNOTHERAPY)

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OBJECTIVE: To study the mode of induction and the mode of action of tumor necrosis factor (TNF) and other antitumor factors present in tumor necrosis serum (TNS).

APPROACH: TNS can be obtained by treatment of Swiss mice with inducers like BCG, C. parvum or zymosan followed by an eliciting injection of endotoxin, a procedure which is lethal for the mouse (Carswell et al. PNAS 72, 3666). To get more information about the induction of TNF and other antitumor factors, sera by variation of mouse strain, inducer, elicitor, etc. will be screened for their in vitro and in vivo activity against Meth A sarcoma cells, for interferon content, colony stimulating activity and other properties.

PROGRESS: Classically prepared TNS inhibits the incorporation of 3H-thymidine into Meth A cells to a significantly greater extent than normal mouse serum (NMS). The in vitro cytostatic activity is not influenced by decomplementation of the sera in vivo before injection of LPS or in vitro. The cytostatic activity is partly heat-labile, partly due to heat-stable non-tumor-specific 'natural' antibodies and other heat-stable factors. Serine esterase inhibitors have no effect. C3H/HeJ mice do not produce sera with enhanced cytostatic activity after combined C. parvum/LPS treatment, while C3H/HeN mice do. Inducers which in combination with LPS can serve for production of strong cytostatic sera comprise both stimulators and blockers of the mononuclear phagocytic system. Elicitors which can substitute for LPS include membrane labilizers and stabilizers. The use of non-LPS elicitors results even in positive C3H/HeJ sera. In vivo the induction of hemorrhagic necrosis by TNS is not decreased by heating decomplementation, inhibition of serine esterases or absorption of antibodies. Many agents can substitute for BCG as an inducing agent, but probably only a few agents can replace endotoxin.

217. STUDIES ON THE ANTITUMOR ACTIVITY OF SYNTHETIC GLYCOLIPIDS

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OBJECTIVE: To develop non-toxic synthetic glycolipid anti-tumor immunoadjuvants and to elucidate their mode of action.

APPROACH: 1. By studying the effect of structurally similar synthetic glycolipids on various arms of the immune response and comparing them with respect to their antitumor potential we would be able (a) to select a glycolipid superior to maltose tetrapalmitate (MTP) in its antitumor action and (b) to reason that stimulation of specific arm(s) of the immune response elicits maximum antitumor activity. 2. By analyzing the lymphocytic population migrating to a tumor after MTP treatment, we may be able to specify which effector cells are involved in tumor regression by MTP. Conversely, homing of tagged effector lymphocytes (monocytes, T and B lymphocytes) obtained from animals with MTP regressed tumors, to tumor sites or to tumor metastasis sites of the same tumor-bearing animals, may indicate the nature of the effector population. 3. To see if primary chemically induced tumors regress or tumor incidence is reduced when animals are respectively treated with MTP after tumor development or from the beginning during cancer induction by a carcinogen. 4. To covalently link modified tumor cells (neuraminidase-treated and iodoacetate treated) to CNBr-activated MTP and to see if intratumoral active immunotherapy with the conjugate will induce tumor regression and prevent tumor metastasis.

PROGRESS: MTP has now been shown to be an effective antitumor immunoadjuvant against a variety of transplantable tumors when the tumor inocculum is small. It has also been found superior to BCG, C. parvum, levamisole and pyran copolymer against one tumor model system (Dunning prostate carcinoma) used in the test. Animals bearing small tumors, after surgical tumor excision, show reduced tumor recurrence if they are treated with MTP. It activates macrophages and elicits release of macrophage stimulating and macrophage chemotactic factors from lymphocytes.

218. IMMUNITY TO TUMOR-LOCALIZED, CHEMICALLY MODIFIED DRUGS

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Bleomycin, macromycin and hematoporphyrin are potent tumoricidal agents that have the unusual property of preferentially localizing on or within malignant cells. Based on work that we have carried out in our own laboratories, together with that done by others, it is clear that bleomycin and macromycin can evoke an immune response, either humoral, cellular, or both depending upon the properties of the carrier species and the route and mode of immunization. Based on methodology that we already have developed, it should be possible to make hematoporphyrin immunogenic. The tumor localizing and cytotoxic properties of these three drugs, each exerted by a different mechanism, suggest that if a sensitized tumor-bearing animal was given the derivatized drug either systemically or intralesionally, the ensuing immune response, directed against the drug, the carrier or the drug-carrier complex, would also be cytotoxic to the tumor cells to which the drug-carrier complex was bound. The specific aims of this proposal are three-fold: 1) To chemically modify three tumoricidal drugs that have tumor localizing properties - bleomycin, macromycin and hematoporphyrin - by linking them to either a lipid-conjugated protein carrier molecule or a lipid so that they become capable of evoking a drug or carrier directed, cell-mediated immune response and to study this by in vivo and in vitro methods. 2) To develop immunotherapeutic models employing these chemically modified drugs. 3) To study the binding affinity that these drugs and their derivatives have for the surface membranes of malignant cells and the possible relationship that this has to their therapeutic effect.

In the immunotherapy model that we propose, the cell-mediated response will be directed against either the unmodified or the chemically modified drug, carrier or drug-carrier complex localized on the surface membranes of malignant cells. It does not depend upon the drug retaining its anti-neoplastic activity although this would be an added advantage.

219. HOMING LIPOSOMES IN CANCER IMMUNOCHEMOTHERAPY

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A new approach to cancer immunochemotherapy is proposd which is particularly appropriate for treatment of metastasizing tumors. Tumor cell-directed liposomes will be used to home chemotherapeutic agents to the tumor target cells. Materials that are enclosed inside liposomes are protected from metabolic degradation and may be selectively concentrated in target cells. Encapsulated agents also may exhibit less systemic toxicity than the free drugs. Homing will be accomplished by attaching antitumor cell antibodies on the exterior of the liposomes. Specific rabbit anti-guinea pig tumor antibodies will be prepared toward the weakly antigenic, syngeneic strain 2 guinea pig line-10 hepatocarcinoma cells. Syngeneic line-1 hepatocarcinoma cells will be utilized as a control for efficacy and specificity. Tumoricidal effects in vitro will be determined primarily by the 51Cr release microcytotoxicity assay. Efficacy in vivo will be tested by various means, including injection of target-directed liposomes (a) together with tumor cells or (b) directly into established tumor nodules or (c) systemically after surgical excision of the primary tumor. Our in vitro experiments demonstrate that liposomes containing both anti-line-10 antibody and actinomycin D specifically home to and kill line-10 tumor cells. This model should be particularly useful in determining the potential for target-directed liposomes in the prevention and cure of tumors metastasizing to regional lymph nodes.

220. EFFECT OF MICROWAVE EXPOSURE ON IMMUNE REGULATORY FUNCTION

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This experiment seeks to investigate the effects of microwave irradiation upon the immune response. This research project is of military and medical importance because of the potential rewards of demonstrating a practical immune stimulant. An immune stimulant would be of use in infectious disease therapy, cancer therapy, biological warfare and during nuclear warfare.

The animal model will be the CD2F1 mouse which will be infected with Maloney leukemia cells on day 0 of the experiment. The CD2F1 mouse supports growth of the MBL-2 tumor cells but ultimately rejects the tumor due to T cell response. In an immunologically suppressed animal the tumor grows uncontrolled and in an animal receiving an immune stimulant drug the tumor regresses more rapidly than in the untreated CD2F1 mouse. We will study the effect upon the immune response of the CD2F1 mouse after infection with MBL-2 cells and microwave irradiation.

221. CONTROL OF NEOPLASTIC DISEASES BY COMBINED TREATMENT WITH IMMUNO-REGULATING FACTORS

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Several agents were found to enhance macrophage and natural killer cell tumoricidal activity (poly ICLC, lentinan, thymopoeitin fractions, MVE, glucan and human chorionic gonadotropin). The enhanced activity was neutralized by interferon antibody, indicating that interferon is the direct activating factor. The tumoricidal activity of interferon may be due to its capacity to enhance macrophage and natural killer cell reactivity to tumor antigens. Prostaglandins E1 and E2 negate macrophage tumoricidal activity via 'shut off' mechanism. TPA, a potent inflammatory and tumor-promoting agent possesses two opposing influences on macrophage reactivity. Conditioned medium from macrophages pulsed with TPA enhanced M109 carcinoma colony formation in vitro. Splenocytes incubated with TPA produced macrophage activating factor. Human chorionic gonadotropin enhanced macrophage and natural killer activity. Human monocyte cultures exposed to human interferon exerted a stronger cytotoxic effect on the Raji lymphoma tumor cell line. Significant adjuvant effect was attained with MVE when it was combined with B16 melanoma vaccine. Increased survival was attained in L1210 leukemia when Azimexon (BM 12.531) and MVE were combined with chemotherapy.

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222. EVALUATION OF CHEMICALLY MODIFIED THERAPEUTIC ENZYMES

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The overall goal of this research is to evaluate various chemical modification procedures for their ability to overcome many of the biological limitations of enzyme therapy such as immunogenicity, rapid clearance, poor biological stability, inadequate pH optima, and poor in vivo distribution. To accomplish this task various amino acid-N-carboxyanhydrides will be polymerized with various enzymes of interest in cancer therapy (asparaginase, carboxypeptidase G, phenylalanine ammonia lyase, and glutaminase) and the above biological properties evaluated. In addition, further preclinical evaluation of poly-D,L-alanyl-asparaginase will continue because these preparations have already shown improved immunogenic, clearance, stability, and therapeutic properties in mice. Also, the in vivo distribution of modified carboxypeptidase G will be evaluated for high dose ip MTX rescue therapy with ascites tumors.

223. OPSONIC AND PHAGOCYTIC ACTIVITY DURING TUMOR GROWTH

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The summary for this project is under entry no. 65.

224. CYTOTOXIC ANTITUMOR ANTIBODIES

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As a means of obtaining more efficient immunotherapy of tumors, we are investigating possible methods for increasing the cytotoxicity of antibodies. Antitumor antibodies have been conjugated to various toxic agents such as radioactive iodine, diptheria toxin, nitrogen mustards and enzymes with subsequent conversion of protoxins to toxins. Selective cytotoxicity has been demonstrated in a number of antitumor antibody-tumor cell models in vitro. These promising systems are being tested in vivo with immunoglobulin-enzyme conjugates in several tumor models in hamsters and mice. Extensive clearance and localization studies with antibodies, antibody fragments and immunoglobulin E are also being conducted to find ways of improving selective tumor localization. Purification of antibody-enzyme conjugates is also being attempted with affinity chromatography techniques.

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225. NATURAL KILLER CELL ACTIVITY DURING INTERFERON THERAPY OF MULTIPLE MYELOMA

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Immunotherapy of cancer with BCG and interferon has been moderately successful in rodents and humans. The mechanism by which these agents cause tumor regression is unknown, but recent findings from our laboratory and others have suggested that both these agents activate natural killer (NK) cells either directly (interferon) or through macrophage intervention (BCG). NK cells spontaneously lyse a wide variety of tumor

cells in vitro. We have shown that mouse NK cells from BCG-stimulated mice are very effective in the suppression of a variety of tumors in vivo. Our main goals, therefore, are to elucidate the mechanism by which BCG and interferon activate NK cells and to relate the activation of NK cells to the regression of neoplasms in rodents and humans during immunotherapy.

The studies proposed involve three interrelated projects. First, we will analyze the cellular and molecular components involved in the activation of rodent NK cells by BCG. We will focus on the role of macrophages, interferon, and prostaglandins in this process. Second, we will extend our recent observations that tumor-bearing mouse NK cells are anergic to stimulation by BCG and that this anergy can be overcome with indomethacin, an inhibitor of prostaglandin synthesis. The regulation of NK activity by soluble factors derived from tumor cells and tumor-bearer leukocytes will be evaluated. Also, the pharmacologic basis for the indomethacin effects will be studied. Third, we will measure blood NK activity levels in human multiple myeloma patients involved in a national interferon trial. We will compare the changes in NK activity with the clinical progress of the patients during interferon therapy. In addition, NK cells from these patients will be stimulated in vitro with interferon and indomethacin to obtain information which may lead to improved therapy regimens.

226. IMMUNOLOGICAL-BIOCHEMICAL RESPONSES IN NEOPLASIA MODULATED BY ADJUVANTS

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The natural killer (NK) activity of an allogeneic tumor system was found to be enhanced by the total splenic population of either pregnant of normal female BALB/c mice treated with human chorionic gonadotropin (HCG) over those values detected for the nonpregnant, untreated controls. The splenic population of mice in the early stage of pregnancy (9-10 days) exhibited a statistically significant increase in NK activity over nonpregnant controls at all effector/target ratios tested. The groups of mice in the mid (12-13 days) and late (15-16 days) stages of pregnancy also showed significantly enhanced NK activity above control values at effector/target ratios of 100:1, 50:1 and 25:1. The i.v. administration of carrageenan into pregnant mice abolished the increased NK activity suggesting a putative role for the macrophage in this system. HCG administered to normal female BALB/c mice resulted in enhanced NK activity similar to that seen for the pregnant mice. This augmented NK activity does not appear to be effected by either the number or timing of the HCG administration. The augmentation of NK activity of HCG-treated mice was also detected in a syngeneic system utilizing 51Cr labeled M109 tumor target cells and BALB/c effector cells.

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227. SELECTIVE MANIPULATION OF T CELL SUBSETS BY ADJUVANTS

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Synthetic adjuvants will be utilized to selectively potentiate T cell subset functions directed against syngeneic tumor cells. These functions will be analyzed using distinct assays such as the generation of cytolytic T lymphocytes against syngeneic tumor cell surface antigens, amplification of the cytotoxic T cell (CTL) response by the helper T cell subset, and the generation of cytolytic antibody directed against syngeneic tumors. In addition, the potential of synthetic adjuvants to generate suppressor T cells which may regulate the activity of cytolytic T cells against tumors in vitro and in vivo will be assessed.

The two models chosen will focus on the period of tumor remission obtained between the time that the primary tumor is identified and the expected onset metastatic disease which occurs as the animals age. Immunotherapeutic protocols using highly activated subpopulations of T lymphocytes together with adjuvants will be tested. In addition, if adjuvants are found to induce suppressor cells in vivo, regimes will be undertaken to circumvent the induction of suppression in vivo. These protocols include thymectomizing the animal, pretreating the animal with cytoxan to reduce the possibility of the induction of suppression, and in vivo administration of anti Ig anti-serum.

These experiments should provide a rational basis for the utilization of adjuvants and specifically activated T cells in tumor-bearing mice.

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228. SELECTIVE STIMULATION OF CELL-MEDIATED CANCER IMMUNITY

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Bovine serum albumin (BSA) heavily conjugated with a lipid (dodecanoic acid) selectively stimulates a type of cell-mediated immunity, delayed hypersensitivity (DTH), instead of the antibody responses usually produced by stimulation with native BSA. The experiments proposed in this project will include consideration of some of the physical, chemical and immunologic properties of chemically modified BSA preparations which selectively stimulate DTH. The cellular basis of the action of these antigens will be studied in vivo and in vitro.

In further experiments, we will prepare and evaluate a series of natural and synthetic materials which may serve as adjuvants to selectively enhance the production of DTH. These experiments hopefully will develop both a rationale and a protocol for stimulating strong, sustained DTH to BSA and other antigens.

Parallel studies will use the information gained from these basic experiments in an attempt to stimulate DTH against purified tumor antigens and to cause regression of established tumors by immunologic methods. Selected tumor antigens will be chemically modified and injected into appropriate animals according to the protocols found most effective in selectively stimulating DTH to BSA. Current studies are directed towards identification of a particularly potent enhancing factor found in the sera of some hamsters bearing SV40 induced sarcomas.

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229. SOUTHEASTERN CANCER STUDY GROUP

Smalley, R. V., Robbins, R., Fischer, S., Vider, M., Neilan, B., Petersen, R., Mayer Scogna, D., Joseph, R., Hanjani, P., Temple University, School of Medicine, Dept. of Internal Medicine, *3322 Broad St., Philadelphia, Pennsylvania,* 19122, United States of America

Support is requested for participation in the cooperative clinical trials of the Southeastern Cancer Study Group. Phase II Studies: Studies, which may or may not be randomized, of the effectiveness of a specific treatment plan in a series of patients with a specific disease or diseases. These studies will be disease oriented. Phase III Studies: Randomized comparisons of two or more treatment plans. Emphasis is now on the combined modality treatment of solid tumors which now accounts for a large proportion of our case accessions. Ancillary and pilot studies alone or with one or more institutions will be performed.

Locally we propose to continue in the use of ancillary and pilot studies including chemotherapy, radiation therapy and immunotherapy. These clinical studies will be performed primarily in patients with solid tumors and will generate data and lead to more extensive groupwide studies. We plan to participate with

the immunotherapy committee in extensive Phase I evaluations of new potential immunostimulating drugs.

Important secondary benefits of these studies are the excellence of patient care and improved training of house officers and medical students.

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B. ACTIVE IMMUNOTHERAPY WITH TUMOR CELLS, TUMOR CELL COMPONENTS, OR OTHER CELLS

230. IMMUNE RESPONSES TO TUMOR CELLS INOCULATED INTO THE GASTROINTESTINAL TRACT

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OBJECTIVE: To investigate the immune responses obtained by inoculating tumor cells into the lumen of the gastrointestinal tract.

APPROACH: C3H mice are immunized by inoculating C3H-L-ascites tumor cells into the lumen of coecum (ic) through a laparatomy or intragastrically through a probe. The immune response is evaluated by transplantation studies or by in vitro studies of cell mediated and humoral mediated immunity.

PROGRESS: Experiments with intracoecal immunization in the model system mentioned above indicate that immunity leads to either inhibition of or enhanced growth of a subsequent graft depending on: 1. the state of the immunizing cells (live cells - inhibition) (frozen-thawed cells -enhancement); or 2. the state of the animals to be immunized (live cells ic into non-treated mice - inhibition) (live cells ic into cyclophosphamide treated mice - enhancement).

In vitro studies have shown that the immunity obtained by intestinal immunization is cell mediated as well as humoral mediated. The humoral immune response consists of tumor specific antibodies and tumor specific factors able to interfere with the binding of antibodies to the cell membrane. These interfering factors lack some of the immunoglobulin determinants. They can not be detected by fluorescein-conjugated anti-mouse globulin.

231. USE OF SYNGENEIC HYBRID CELLS IN LEUKEMIA THERAPY

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Somatic hybrids of ASL-1 murine leukemia cells and LM(TK) cells, a sustained mouse cell line, express tumor antigens immunologically cross-reactive with a similar tumor associated antigen formed by ASL-L cells. Although the hybrids are neoplastic in immunologically deficient animals, they are rejected after forming a localized tumor in imm nocompetent syngeneic (A/J x C3H/HeJ)F1 mouse recipients. Mice rejecting hybrid cell tumors are partially and in some instances completely resistant to otherwise lethal injections of the leukemia cell line used as one parent in forming the hybrids. One hundred percent of animals not injected previously with ASL-1 x LM(TK)- hybrid cells die of leukemia if injected with ASL-1 cells.

I propose to investigate the use of syngeneic hybrid cells in the treatment of spontaneously occurring and passively transferred murine leukemia.

Somatic hybrids of ASL ı and LM(TK)- cells are prepared with the aid of polyethylene glycol. Fused cells selected in medium containing hypoxanthine, aminopterin and thymidine (HAT) may be cultivated indefinitely in vitro. They share the histocompatibility antigens of both parents and a hybrid karyotype. Cellular immune mechanisms are involved in the resistance of mice rejecting hybrid cells to the successful transfer of ASL-1 cells. Cells from the spleens of mice rejecting hybrid cells inactivate ASL-1 leukemia cells in vitro and stimulate the release of 51Cr from prelabeled ASL-1 cells. The reaction is specific for the leukemia cell line used as one of the parents in forming the hybrid. Syngeneic mice receiving ASL-1 cells simultaneously with or followed by ASL-1 x LM(TK)- hybrid cells survive for significantly longer periods than mice receiving ASL-1 cells alone. This finding is of especial interest because it occurs entirely in an inbred mouse system in which histocompatibility differences between leukemic and hybrid cells are likely to be confined to immunologically crossreactive tumor-associated antigens expressed by neoplastic leukemia cells and by non-neoplastic viable hybrid cells.

232. IMMUNOMODULATION OF ONCOGENESIS

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The overall goal of this study is to determine the biological significance of a sub-optimal anti-tumor immune response.

The role of the immune system in regulating tumor growth has been controversial since the demonstration that tumors carry neoantigens recognizable by the host. This controversy is particularly active with respect to the autochthonous or primary-tumor-bearing host. It seems appropriate, therefore, to study the influence of the immune system on tumor induction and progression in the primary host, along with the progression of neoplasms from benign to malignant, using a tumor transplantation system.

Immunomanipulated mice will be used to determine whether a weak, but specifically directed, anti-tumor response will induce a higher than normal probability of tumor development over that seen in mice with an immune responsiveness that is either much stronger or essentially absent. The tumors induced in these experiments will then be tested to determine whether the immunologic status of the host was able to modify the tumor's immunogenicity.

Preliminary results have documented that animals with a suboptimal immune capacity developed more skin tumors with shorter latent periods than those animals with either a greater of lesser immune capacity. If the immune system can stimulate as well as inhibit the induction and progression of neoplasia, the need for defining the biological boundaries of these effects becomes essential for development of effective immunotherapy protocols.

233. MECHANISMS OF VIRAL ADJUVANCY IN IMMUNITY TO TUMORS

Zinkernagel, R. M., Rosenthal, K. L., Scripps Clinic & Research Foundation, Dept. of Cellular & Developmental Immunology, *476 Prospect St., La Jolla, California*, 92037, United States of America

The studies described in this proposal are aimed at analyzing the effect virus infection of tumor cells has on the immunogenicity of these tumor cells and attempting to define cellular immune mechanisms involved in the improved immune response possibly directed against tumor-specific cell surface antigens. We intend to assess the immune responses in vitro by measuring cytotoxic T cell activity and/or T helper activity towards both tumor-specific antigens as well as virus-specific determinants. Furthermore, the role of major histocompatibility determinants in T cell-mediated immunity and its enhancement will be studied and evaluated with respect to requirement and function in the enhancement of immunogenicity of tumor associated antigens by virus-infection. The results will be evaluated in vivo for biological relevance with respect to both protection and specificity. These studies should not only provide a better understanding of the viral adjuvance effect but also give a better characterization of the in vivo effects and thus further our understanding of cellular anti-tumor effector mechanisms of the vertebrate host.

234. SOLUBILIZATION AND PURIFICATION OF TUMOR-SPECIFIC TRANSPLANTATION ANTIGENS

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Distinctive antigens on the surface of tumor cells may provide the stimulus for the host resistance against neoplastic progression. Purification of the immunoprotective principle in 3M KCl extracts of a methylcholanthrene sarcoma in C3H mice by isoelectric focusing yielded Fraction 15 of pI 5.95, 55,000

approximate molecular weight which was devoid of H-2 determinants and of tumor growth facilitating activity. In the past year administration of Fraction 15 after inoculation with tumor was demonstrated to retard the outgrowth of a 1) primary challenge, 2) local metastasis, and 3) recurrence after surgical resection. A new extraction technique utilizing single phase butanol was demonstrated to have several advantages over 3M KCl: (1) It yields Fraction 15 directly from cells without releasing tumor growth facilitating moieties. (2) It is devoid of H-2 activity. (3) It is not heavily contaminated with cytoplasmic proteins. (4) The yield and specific activity of the immunoprotective antigen is 20 fold greater than 3M KCl extracts. (5) The tumor cells are viable after the treatment (noncytolytic cell surface extraction), thus affording the possibility of multiple extraction cycles. The proposed experiments will utilize butanol extracts for active specific immunotherapy of local recurrence after surgical resection both of transplantable neoplasms and of subcutaneous tumors induced in primary hosts with methylcholanthrene. In addition to these clinically oriented studies, there will be continued investigations to develop new extraction methods, to analyze host responses upon stimulation with extracts, and to dissect the molecular basis of tumor antigen polymorphism.

235. STUDY ON REACTIONS OF TUMOR ANTIGENS WITH THEIR ANTIBODIES

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This is ongoing research with a major objective of developing immunodiagnostic and immunotherapeutic procedures in malignancies. Considerable effort has been devoted to studies on carcinoembryonic antigen (CEA). The diagnosis of tumor based on demonstration of this antigen in feces has been, however, complicated by the appearance of CEA in normal GI mucosa and in food products. Immunization of rats and mice with an antigen analogous to human CEA is being energetically pursued. The appearance of newly discovered pancreas-specific antigen in duodenal contents and feces is being studied as a possible tool for the early diagnosis of pancreatic carcinoma. Formation of antibodies directed against tumor-specific cell-surface antigens (TSCSA) has been studied by means of several sensitive procedures, and diagnostic application of detection of such antibodies is being explored. Studies on genetic control of immune responses to TSCSA have been initiated. Special attention is devoted to antigens shared by tumor cells and normal allogeneic and xenogeneic cells. This includes extensive studies on heterophile antigens, primarily Paul-Bunnell antigen. This antigen was shown to appear not only in the course of infectious mononucleosis, but also in lymphoma and leukemia. The immune response to this antigen appears to be of great importance in the elimination of transformed, aberrant cells. Adsorption by tumor cells of erythrocytes sensitized by IgG antibodies is being explored as an intriguing biological phenomenon and a possible diagnostic procedure. Tumor growth-promoting activity of circulating immune complexes unrelated to the tumor antigens is also being investigated.

236. SELECTIVE STIMULATION OF CELL-MEDIATED IMMUNITY

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Immunochemical studies performed during the last decade on well characterized antigens revealed that antigens may contain areas recognizable by B cells, T cells, and by both. Using several model antigens it is proposed to selectively activate T cells by blocking the B cell recognizable areas with antibodies. It is thus hoped to ascertain and elucidate the selective induction and elicitation of cell-mediated immunity by antigen-antibody complexes.

237. MECHANISMS OF TUMOR REGRESSION BY HAPTEN TREATMENT

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The purpose of this investigation is to study the in vivo mechanism of tumor regression following immunotherapy using a hapten-modified tumor tissue system. Preliminary work has shown that hapten modification of target tumor by conjugation with L-phenylalanine mustard followed by intravenous administration of antiserum raised against this hapten conjugated with appropriate tumor extract will cause complete regression of both allogeneic and syngeneic rat tumors. In experiments with either allogeneic or syngeneic tumor a single treatment of one of the two bilateral tumors causes complete regression of both. Results indicated that this effect was due to neither PhM alone nor antiserum alone. We consequently found that antiserum against PhM conjugated-carrier protein unrelated to the target tumor was equally effective, thus indicating that both the hapten and antihapten antibody have a role in tumor regression. Histological observation suggested that the reaction may be similar to the Arthus reaction. We will determine the mechanism of tumor regression utilizing syngeneic rat tumor and an active fraction of syngeneic and xenogeneic anti-PhM serum. As a first step, in vitro cytotoxicity of anti-PhM antibody on PhM-treated tumor cells with or without complement will be determined. Similarly the role of polymorphonuclear leukocytes in this regression will be studied. In addition a non-oncolytic hapten (dinitrophenol) will be studied in the same model in order to compare effects.

238. MECHANISM OF NANAASE-ENHANCED TUMOR IMMUNOGENICITY

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Immunization of tumor-bearing hosts with Vibrio cholerae neuraminidase (NANAase)-treated tumor cells alone or in combination with chemotherapy slows the growth or causes regression of established tumors. Although this type of immunotherapy has been explored in a number of animal models and has been used in man, information is lacking on why NANAase treatment enhances immunogenicity. We have developed a chemoimmunotherapy model for treating L1210 ascites tumors in DBA/2J mice by combining 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosurea (MeCCNU) with NANAase-treated purified L1210 plasma membranes. Because the immunogen is a purifed subcellular particle of known protein and glycoprotein composition, we can analyze at the molecular level the NANAase modifications of the membranes and how these affect the immune response to the tumor. We will use two-dimensional immunoelectrophoresis to analyze the immune response of DBA/2J mice to NANAase-treated L1210 plasma membranes to determine the relationship between glycoproteins modified by NANAase and the antigens to which the host responds. We will also determine whether the plasma membranes of tumor cells surviving chemotherapy are more closely related to NANAase-treated membranes prepared from the parent tumor than to untreated membranes from that tumor. This will be done by producing an L1210 tumor line resistant to MeCCNU chemotherapy and by using biochemical and immunochemical techniques to compare its plasma membranes with those of NANAase-treated and untreated membranes of the parent tumor. In addition, we will study the role in the immune response to NANAase-modified membranes of naturally occurring serum factors that recognize determinants on NANAase-treated cells; we will determine whether modified membranes are more effective in animals preimmunized to increase the level of these factors or whether modified membranes preincubated with serum containing these factors are more effective than those that have been preincubated.

239. IMMUNOTHERAPY OF BOVINE OCULAR SQUAMOUS CARCINOMA

Tompkins, W. A., University of Illinois, Urbana-Champaign Campus, Graduate School, *Urbana, Illinois*, 61801, United States of America

OBJECTIVE: BOSC is a serious disease causing major economic losses in the livestock industry. This study will examine the efficacy of immunotherapy for BOSC. Experiments will be designed to detect tumor associated antigens on the surface of BOSC cells and determine if BOSC is capable or responding immunologically to these antigens. Antibody, T-cell and non-

specific lymphocyte cytotoxicity against the BOSC cells will be tested in vitro by a Cr cytotoxicity assay.

APPROACH: After obtaining baseline information on the immune response to BOSC antigen we will develop immunotherapy protocols to augment the immune response and cause tumor regression. Corynebacterium parvum, cryosurgery and extracted tumor antigens will be used as immunotherapy agents. Field trials correlating tumor status with immune response to BOSC tumors will be done for each immunotherapy protocol.

240. CANINE CHEMOIMMUNOTHERAPY STUDY

Theilen, G., University of California, Davis Campus, Agricultural Experiment Station, Depts. of Surgery/Medicine, *Davis, California*, 95616, United States of America

OBJECTIVE: This study will test a number of different combinations of chemotherapeutic and immunotherapeutic regimes on dogs with osteosarcomas or lymphosarcomas and make recommendations on those showing the greatest potential for clinical use.

APPROACH: Obtain from biopsies tumor cells which will then have antigens on their cell surfaces altered by chemicals. The altered cells will then be injected as a vaccine along with other chemotherapeutic agents. Animals will be followed for 1, 3 and 5 years post-treatment to determine success.

241. ACTIVE IMMUNOTHERAPY AND IMMUNE MEDIATED REGRESSION OF BOVINE OCULAR CARCINOMA

Pierce, K. R., Joyce, J. R., Pack, F. D., Texas A & M University, College Station Campus, School of Veterinary Medicine, Dept. of Veterinary Pathology, *College Station, Texas*, 77843, United States of America

The summary for this project is under entry no. 185.

242. IN VITRO SELECTION OF LYMPHOCYTES WITH SPECIFIC ANTITUMOR CYTOTOXIC ACTIVITY

Colnaghi, M. I., DellaPorta, G., Menard, S., Istituto Nazionale per lo Studio e la Cura dei Tumori, Division of Experimental Oncology A, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: To verify whether the supernatant from Con A-treated murine spleen cell cultures which induces a continuous growth of murine lymphocytes from an unidirectional mixed cell culture and selects progressively T cells specifically cytotoxic for the stimulating cells has a species specific activity. The selection of a lymphoid cell population specifically directed against a certain antigenic specificity would allow immunotherapy experiments as well as the characterization of tumor specific antigens.

APPROACH: The supernatant from Con A-treated murine spleen cell cultures will be added to human lymphoid cells recovered from various types of mixed cultures and the possible establishment of a continuous line with a specific cytotoxic reactivity will be verified.

243. ROLES OF ARMED MONOCYTES IN CONTROL OF METASTASIS

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This project is designed to investigate the roles of monocytes in limiting blood-borne metastasis of tumor cells, and to provide a valid basis for effective immunochemotherapy (or prophylaxis) of metastatic spreading.

Utilizing both metastasizing and nonmetastasizing syngeneic tumor lines of DBA/2J mice, host immunity is semi-quantitatively assessed in normal and tumor-bearing animals by injection of radio-labeled tumor cells and by measuring the rate of tumor cell destruction in various target organs of these animals, and the results are evaluated in relation to eventual tumor outgrowth in each organ.

Responses of tumors to chemotherapy and the metastasizing ability of tumor cells are also comparatively investigated.

Suppression by commonly employed chemo- and/or radiotherapeutic regimens of host defense functions and their possible restoration or augmentation by monocytic effectors are studied. Possible roles of T lymphocytes in such monocyte functions, as well as roles of monokines in activation of T cell activities, are investigated.

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C. ADOPTIVE IMMUNIZATION

1. MONOCLONAL AND OTHER ANTIBODIES

244. SPECIFIC IMMUNOTHERAPY WITH MONOCLONAL ANTIBODIES

Bast, R. C., Ritz, J., Knapp, R. C., Sidney Farber Cancer Center Inc., *44 Binney St., Boston, Massachusetts*, 02115, United States of America

Using somatic cell hybridization techniques, monoclonal antibodies have been prepared against two human malignancies, acute lymphoblastic leukemia (ALL) and ovarian carcinoma. Studies are proposed to examine the pharmacokinetics and toxicity of these reagents after intravenous or intraperitoneal infusion. Methods will be sought to utilize or to prevent antibody-induced modulation of antigens from the surface of ALL cells. Monoclonal antibodies will be used to eliminate leukemic cells from bone marrow before antigenic modulation occurs, facilitating effective autologous marrow transplantation. Attempts will be made to analyze the capacity of antibodies of different subclasses to activate cellular or humoral effector mechanisms. The immunostimulant C. parvum will be administered to augment cellular effector function for antibody dependent tumor cell lysis. These studies should help to provide a more rational basis for the use of monoclonal antibodies for serotherapy of human cancer.

245. MONOCLONAL ANTIBODY THERAPY OF CANCER

Bernstein, I. D., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington*, 98104, United States of America

With the development of hybridization techniques for the production of antibody-producing cells it is now possible to obtain virtually unlimited quantities of monoclonal antibodies directed against defined antigens. During the past year we have established twenty independent hybrid cell lines that produce monoclonal antibodies against antigens of leukemia viruses. The purpose of this proposed study is to evaluate the use of these monoclonal antibodies for the therapy of neoplastic disease in mice.

Specifically, we will continue to isolate a series of hybrid cells that are producing monoclonal antibodies against the proteins of mouse leukemia virus and leukemia-associated antigens. Therapeutic potential of these antibodies in the treatment of transplanted and spontaneous leukemias and sarcomas will then be evaluated. The class of immunoglobulin and specificity of the monoclonal antibodies, as well as their interactions with host factors (complement components, lymphocytes, macrophages) in the tumor rejection phenomena, will be determined.

246. USE OF MANIPULATION OF IMMUNE RESPONSES TO EMBRYO-ASSOCIATED ANTIGENS IN THE IMMUNOTHERAPY OF LOCAL AND WIDESPREAD MALIGNANT DISEASE IN MICE

Gorczynski, R. M., Khomasurya, B., MacRae, S., Ontario Cancer Inst., Division of Biological Research, *500 Sherbourne St., Toronto, Ontario*, M4X 1K9, Canada

OBJECTIVE: We are interested in exploring further the role of embryo-associated antigens in the immune response to spontaneously appearing murine adenocarcinomas.

APPROACH: To date our evidence points to developmental-stage unique embryonic antigens as playing a role in immune sensitization or the regulation thereof, in an experimental system in which we measure subcutaneous growth of these tumors.

As a way of examining the embyronic antigens themselves in more detail we intend to immunize C3H/HeJ mice with crude embryo antigen preparations, and prepare monoclonal antibodies (from the spleen of these animals) by fusion with the tumor line P3X63Ag8. These antibodies will be used not merely to characterize the antigens of the tumor cell itself, but also to manipulate the response within the host of origin of that tumor.

Before attempting the latter, however, it will be necessary to know more about the cellular interactions involved in regulating the response to embryo-associated antigenic determinants, and how these interactions can be manipulated in other model systems. To this end we are continuing to investigate idiotype-anti-idiotype regulation of antibody responses to soluble protein antigens, and of cytotoxic responses to alloantigens. In addition, we are analyzing the role of the host 'thymic environment' in controlling the receptor repertoire for responses to xeno-, allo-, and syngeneic (especially embryo-associated) antigenic determinants.

247. ANTIBODIES AS POTENTIAL TARGETING AGENTS IN PROSTATIC CANCER

Heston, W. D., Washington University, School of Medicine, Dept. of Surgery, *660 S. Euclid Ave., St. Louis, Missouri,* 63110, United States of America

Present attempts to increase cytotoxic agents' selectivity for tumor tissue include binding of the cytotoxin to antibodies which are specific for the tumor. The purpose of this proposed investigation is to examine the usefulness of antibodies directed against normal tissue-specific antigens of non-vital tissues, i.e., prostate, either singly or in conjugation with antitumor antibodies in destroying prostatic carcinoma cells. This investigation will examine the distribution and cytotoxicity of antibodies against prostate-specific acid phosphatase, prostate steroid binding protein, and other prostate tissue-specific antigens. The amount of specific uptake of these antibodies will be determined in vivo by normal prostatic tissue in the rat and dog and in prostatic tumors in the rat. The amount of tissue-specific antigen uptake will also be determined following injection of antisera directed against cell surface antigens. In addition to standard methods of antibody production and in order to generate the amount of antibody required for chemotherapeutic targeting, we will use myeloma fusion with cells extracted from spleens of antigen sensitized mice. This may prove useful with monoclonal antibody.

In in vitro experiments with rodent and human prostatic tumors, we will examine complement and cell mediated tumor cell lysis in the presence of antisera by the use of chromium release procedures.

Initial tumor targeting will be performed with non-covalent adsorbed chlorambucil. The effects of this adsorbed material will be examined both in vivo and in vitro.

248. ANTIBODY-LINKED CYTOTOXIC AGENTS IN THE TREATMENT OF CANCER

Ghose, T. T., Blair, A. H., Vaughan, K., Dalhousie University, Dept. of Pathology, *Halifax, Nova Scotia,* B3H 4H7, Canada

Our objective is to use anti-tumor antibodies as carriers of cytotoxic agents leading to preferential localization in tumors and selective destruction of malignant cells. This project stems from our demonstration that chlorambucil-linked anti-tumor globulins caused greater tumor inhibition in vivo and in vitro than antibody or drug alone.

The aims of this proposal are: (1) Develop a wider range of methods for binding different anti-cancer drugs to immunoglobulins; (2) improve the specificity and purity of carrier antibodies; (3) enhance localization of drug-antibody conjugates at target sites; and (4) assess tumor inhibition by conjugates in clinically pertinent models, e.g., animals with micrometastases and human tumors in nude mice.

Tumors vary in sensitivity and may acquire resistance to a given agent. Therefore, it is important to learn which agents will be active when administered as antibody conjugates and which chemical linkage will give greatest tumor inhibition. This should identify optimal drug-linkage combinations and develop guiding principles for this approach to cancer therapy.

Agents under study are adriamycin, which binds to DNA, alkylating agents, e.g., trenimon and chlorambucil, and methotrexate, an anti-metabolite. Coupling methods include both direct covalent and non-covalent linkages between drug and antibody and linkage via spacers, e.g., polylysine and dextran. Having demonstrated that progressive localization of antibodies against tumor-associated transplantation antigens (TAAs) depends upon the content of specific antibody, we are continuing efforts to obtain preparations enriched in anti-tumor globulins. Methods include: (1) Initial immunization with TAA-enriched fractions, (2) affinity chromatography of antisera, and (3) production of monoclonal antibodies against TAAs.

249. HYBRIDOMA DERIVED MONOCLONAL ANTIBODY FOR EXPERIMENTAL AND CLINICAL CANCER THERAPY

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The summary for this project is under entry no. 155.

250. PRODUCTION AND IN VIVO LOCALIZATION OF TUMOR SPECIFIC ANTIBODY

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OBJECTIVE: To produce tumor-specific antibody by the method of B cell hybridization with a myeloma cell line and to study the factors which determine localization of the antibody in animal tumors. To obtain a localization suitable for the purpose of carrying scanning or therapeutic agents to the tumor.

APPROACH: Hybrid cells are produced by fusion of spleen cells with the P3-20 mouse myeloma cell line derived from MOPC21. The spleen cells are taken from mice which have been immunized with tumor cells. Clones of hybrid cells are tested for the production of tumor-specific antibody and large quantities of antibody can be produced by growing the hybrid cells in culture or in mice. The distribution of 125I-labelled antibody in the tumor and different tissues is to be investigated for different animal tumors and conjugates of antibody and drugs. Degradation of antibody and shedding of antigen and antigen-antibody complexes by the tumor cells interferes with localization. The in vitro study of this inactivation of antibody is expected to provide information about drugs which could be used in conjunction with, or bound to, antibody to give an improved localization. The therapeutic effectiveness of 211 At- and 131I-antibody will be investigated.

PROGRESS: Spleen cells from DBA mice immunized against the L5178Y mouse lymphoma have been fused with myeloma cells and specificity testing of the immunoglobulin produced by some clones is under way. It has been found that xenogeneic antibody bound to L5178Y cells through the Fc or Fab portion is to a large extent degraded or released from the cells as complexes. This inactivation of antibody was shown to limit severely the localization of a highly purified antibody in tumors.

251. IMMUNOPREVENTION AND IMMUNOTHERAPY OF CANCER IN EXPERIMENTAL ANIMALS

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Tumor cell and viral vaccines successfully prevented transplantable and carcinogen-induced cancers in rats. Passive immunity processed as murine-specific IgG successfully prevented spontaneous leukemias and chemically (3-MC)-induced tumors in mice. Endogenous retroviruses derived in high titer (10 to the 11th power virus particles per ml) from various strains of mice (AT124, M55, C57L) and grown in human and canine cells, and baboon virus (grown in canine and human cells) administered intravenously or intraperitoneally successfully eliminated existing cancer, including multiple metastatic cancers in dogs and (preliminary) in cats. This radical new approach to viral immunotherapy of a variety of cancers in dogs and cats will be extended to rabbits and primates bearing cancers.

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252. IN VIVO ROLE OF ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY

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A model is used in which 51Cr-labeled Ehrlich ascites tumor (EAT) cells are injected intraperitoneally (i.p.) into A/J mice and elimination of radioactivity is measured by whole body counting. Mice eliminate 51Cr rapidly during the first 24 hours and at a much slower rate thereafter. This double pattern of elimination is due to the fact that 51Cr labels mainly small (less than 13,000 MW) intracellular molecules which the mouse rapidly eliminates whereas larger labeled molecules are slowly eliminated. When 51Cr-EAT cells are injected i.p. with mouse anti-EAT antibody, the rapid phase of elimination of 51Cr is significantly accelerated. Antibody-coated tumor cells recovered from mice one hour after injection are damaged as manifested by increased susceptibility to osmotic lysis. The number of tumor cells recovered from the peritoneal cavities of mice is higher in mice receiving normal serum than in those receiving antibody. Furthermore, mice inoculated with 51Cr-EAT cells alone die of tumor whereas those receiving antibody survive. Complement is not required since serum from A/J mice does not lyse antibody-coated EAT cells and mice treated with cobra venom factor eliminate 51Cr from antibody-coated tumor cells as fast as normal mice. Mice previously stimulated i.p. with proteose peptone can lyse a larger tumor load than normal mice, whereas silica-treated animals are unable to induce cytolysis of antibody-coated tumor cells. Finally, the ability of anti-EAT antibody to induce accelerated clearance of label was unimpaired by lethal irradiation of the mice. These experiments show that in this model complement-independent antibody-dependent cytolysis can occur in vivo and participate in the rejection of EAT cells.

253. PHASE II PROTOCOL FOR THE TREATMENT OF MALIGNANT LYMPHOMAS WITH ANTI-THYMOCYTE GLOBULIN

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As of March 1980, 8 patients have entered this protocol. All had advanced disease unresponsive to multiple chemotherapeutic agents. Among these 8 patients, there were 3 partial responses which lasted from one month to eight months. Two patients showed no response. Three patients died before response could be evaluated. These 3 patients all died of infectious complications primarily related to their underlying diseases previously reported to this committee. One patient experienced a development of serum sickness which caused discontinuation of the treatment. There has been considerable toxicity from this protocol including the development of infections in 3 patients. All of them died from their infections. In addition all patients developed low grade fevers, several patients developed shaking chills. The preliminary conclusions are that this form of immunotherapy does cause partial remission in patients, however it is extremely toxic. Plans are to continue the study until two other patients with advanced disease have been studied.

254. CONTROL OF NEOPLASIA BY PASSIVE SERUM THERAPY

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The overall research program proposed here is designed to determine the effectiveness of passive serum therapy in the control of malignant growths. In order to broaden the applicability of the results obtained, several tumor systems are being explored (leukemia, sarcoma, adenocarcinoma) primarily utilizing inbred mice. Special attention is being devoted to two aspects of this problem. First, we are seeking to devise the most efficient and safe protocols by judiciously choosing our target antigens and the respective antibodies. Secondly, the underlying mechanisms responsible for successful tumor control or rejection are being actively investigated.

In an attempt to bridge the gap between experimental and human tumor systems, we have embarked on a project which seeks to establish a cross-reactivity between primate viral antigens and those associated with certain human malignancies. We will also search directly for antigens and antibodies which are tumor specific in man. For this, we have chosen a poorly studied but highly lethal human lung tumor (lung carcinoma) and are proposing a novel approach to identify such entities.

255. MECHANISMS OF THE PASSIVE SERUM THERAPY OF MURINE LEUKEMIA

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The objective of this research project is to extend previous work which has established that mice can be protected against Friend leukemia virus (FLV)-induced disease by administration of heterologous antisera raised against disrupted FLV or the purified major envelope glycoprotein of FLV, gp71. Results obtained thus far suggest that passive therapy with the specific antisera acts to reduce the virus burden below the immunosuppressive threshhold, allowing the infected host to develop an anti-viral immune response which, in turn, leads to long-term protection against the FLV infection. Previous studies have also demonstrated the efficacy of the serum therapy protocol in nude athymic mice and preliminary adoptive transfer experiments and the utilization of agents inhibiting macrophage functions suggests that neither host T-cells nor macrophages play a critical role in the serum-protection mechanism. However, detailed investigations will be needed to establish the nature of the host immune response (especially cellular) which is involved in the passive serum therapy of FLV leukemogenesis, and in defining the specific immunologic mechanism(s) resulting in this protection. Those investigations form the basis of the ongoing work and include an analysis of the ability of immune cells from serum-protected mice to adoptively transfer anti-FLV resistance to irradiated unimmunized recipients, as well as an identification of the cell populations involved. The generation of virus-specific cytotoxic effector cells and cells susceptible to blast transformation in vitro by purified FLV gp71 will be monitored, and blast transformation and plaque-forming cell assays with viral, T-dependent, and T-independent antigens will be used to examine the effects of serum therapy on the characteristic development of FLV-induced immunosuppression, as well as to determine whether the regulation of helper and/or suppressor cell populations may be responsible for serum protection. In vitro assays will be utilized to investigate the possible role of antibody-dependent cellular cytotoxicity (ADCC) reactions in protection and possible interactions between passively administered antibodies and hosts' immune cells will be examined by means of pre-protection experiments in which the anti-viral antibody treatment is terminated far in advance of virus challenge. (Text Abridged.)

256. EXPERIMENTAL STUDIES ON IMMUNITY TO CANCER CELLS

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Work on passive immunotherapy of tumors with heterologous antisera of 'directed' specificity is planned to progress from work with long-transplanted lines of L1210 to work with newly derived tumors. These include spontaneous and radiation-induced lymphomas, sarcomas (radiation-induced, dimethylbenzanthracene-induced, and Moloney sarcoma virus-induced), and carcinomas (dimethylbenzanthracene-induced). The cellular dynamics of the therapy process will be studied by methods that will focus on the whole animal, rather than on in vitro work with restricted numbers of interacting cell populations. Inclusion of chemotherapy, injection of complement and use of membrane-active enzymes are included in the protocol. All work with newly derived tumors will be done in C57BL/6J mice.

2. IMMUNOCOMPETENT CELLS

257. ADOPTIVE IMMUNOTHERAPY OF CANCER BY ALLOGENEIC MARROW

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We have developed two distinctly different approaches for successfully transplanting bone marrow across the H-2 barrier in mice without the complication of GVH disease. The first approach involves selectively deleting lymphocytes with specific GVH reactivity from the donors' marrow by diverting the traffic of these cells elsewhere (Fed. Proc. 37: 591, 1978). The second involves using heterologous anti-lymphocyte sera (ALS) plus supralethal irradiation to condition the marrow recipients. With both approaches successful engraftment and the induction of hematopoietic chimerism was to a great extent dependent upon the dose of marrow transplanted. Chimeric mice almost invariably permanently sustained skin allografts of marrow donor haplotype whereas non-chimeric mice did not. When 1 X 10 to the 7th power C57BL/6 marrow was transplanted to supralethally irradited AKR mice, the long-term survivors that were chimeric did not develop spontaneous leukemia-lymphomas. Non-chimeric long-term survivors had a high incidence of leukemia-lymphomas, as did normal untreated mice. We plan to employ both approaches to use allogeneic bone marrow in combination with chemoradiotherapy to treat the spontaneous leukemia-lymphomas that arise in AKR and SJL mice. We also plan to use both approaches to transplant marrow across the major histocompatibility complex in mongrel dogs.

258. SPECIFIC ADOPTIVE IMMUNOTHERAPY OF AKR LEUKEMIA

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The overall objective of the proposed research is to gain greater understanding of the principles involved in the generation of allosensitized cytotoxic effector cells as they relate to tumor and allograft immunity. The main focus of the proposed studies will be: 1) To study allosensitization of CBA mice as a means to generate cytotoxic effector cells against AKR leukemia. 2) To investigate the nature of the antigenic target on AKR leukemia cells which enables effector cells from allosensitized, histocompatible donors to kill them. 3) To investigate the effector mechanism(s). 4) To transplant effector cells from allosensitized, histocompatible donors for adoptive immunotherapy of AKR mice bearing advanced spontaneous leukemia.

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259. GERM-FREE TECHNIQUES APPLIED TO ADOPTIVE IMMUNOTHERAPY

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The overall objective of this research is to develop an effective treatment plan using adoptive immunotherapy in combination with chemoradiotherapy for spontaneous leukemia in AKR mice. In particular, the beneficial effects of bacterial decontamination and isolation in a protected environment are being studied.

Procedures for elimination of bacteria from the intestinal tract have been successfully applied to AKR mice after diagnosis of spontaneous acute T cell leukemia. Leukemic mice are rendered bacteria-free by continuous treatment per os with high dose antibiotics; a sterile laminar airflow environment is used to protect decontaminated mice from exogenous microorganisms. Leukemia remission induction with chemotherapy is essential to keep a significant proportion of leukemic mice alive during decontamination. For hematopoietic support and adoptive immunotherapy, mice undergoing decontamination are injected with bone marrow cells from conventional H-2 compatible or incompatible donor mice. Adoptive immunotherapy when applied to decontaminated leukemic AKR mice in remission can result in significant long-term leukemia-free survival without apparent secondary disease. Leukemia-free chimeras can be returned to the conventional environment. Experiments using the experimental treatment model to answer important questions concerning the clinical applicability of this model are continuing.

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260. ADOPTIVE CHEMOIMMUNOTHERAPY OF TUMORS IN MICE

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Several models have been developed in the applicant's laboratory for the eradication of advanced syngeneic antigenic murine leukemias and sarcomas by a combination of non-curative chemotherapy and adoptively transferred lymphoid cells. The objective of the proposed research is to further study the prerequisites for efficacy of such 'adoptive chemoimmunotherapy' with syngeneic and allogeneic lymphoid cells and explore some of the problems posed by those prerequisites so as to lend rational direction to eventual attempts at a similar immunotherapy in man. The principal problems to be investigated will be the following: 1) The antitumor reactivity of mice bearing leukemia and the influence of therapy on such reactivity, 2) the sensitization of lymphoid cells to tumor antigens in vitro and their immunotherapeutic efficacy in vivo, 3) the therapeutic use of lymphoid cells 'armed' in vitro with specific antiserum, 4) prevention and treatment of graft-versus-host disease (GVHD) by 'hot pulse suicide' techniques in vitro and immunosuppressive agents in vivo, and 5) the use of GVHD preferentially or specifically against the leukemia. It is hoped that the availability in the same laboratory of effective and reproducible models for adoptive chemoimmunotherapy and for induction of fatal GVHD in vivo and assays for cell-mediated immunity to tumor and non-tumor antigens in vitro will make it possible to attain those objectives.

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261. SENSITIZATION TO POOLED ALLOGENEIC CELLS - A NEW APPROACH TO INDUCTION OF TUMOR IMMUNITY

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The overall objective of the proposed research is to determine whether in vivo immunization of mice with pooled allogeneic normal cells representing many different H-2 haplotypes or whether adoptive transfer of in vitro 'pool' sensitized cytotoxic T lymphocytes (CTLs) confers immunity to tumors both in terms of tumor prevention and immunotherapy of residual tumor cells. The rationale for this proposal is based on findings that (1) Tumor cells express antigens cross-reactive with allogeneic histocompatibility antigens and (2) human and mouse lymphocytes sensitized with a pool of normal allogeneic cells will lyse autologous and syngeneic transformed cells, respectively, in vitro. We propose first to test the efficacy of in vivo immunization of mice with pooled cells in terms of inducing resistance to challenge with tumor cell lines or development of primary tumors and metastases. Secondly, we propose to stimulate mouse lymphocytes in vitro with the pool and determine whether the CTLs generated are directed against tumor associated tranplantation antigens. Thirdly, the in vitro generated CTLs will be tested in vivo for their ability to (a) prevent outgrowth of admixed tumor cells, (b) adoptively transfer immunity to tumors and (c) prevent metastases.

262. MECHANISMS OF IMMUNE ERADICATION OF TUMORS

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This project concerns the interaction of specifically sensitized spleen lymphocytes and tumor cells. Efforts were focused on correlating the results of in vivo cytotoxicity tests with in vivo tests of tumor cell killing. The results indicate that the cell populations responsible for in vitro cytotoxicity are separable from the cell population responsible for adoptive transfer of immunity. These findings indicate the need for confirmation of results of in vitro experiments in animals. Cells mediating adoptive transfer of immunity do react with the targets in vitro, but this interaction does not lead to target cell destruction.

263. ANTI-TUMOR POTENTIATION AND SPONTANEOUS LYMPHOMAS IN THE MURINE GVH SYSTEM

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The graft-versus-host (GVH) reaction, induced by the injection of parental strain lymphoid cells into genetically tolerant F1 hosts, induces a marked degree of immunosuppression by day 7 which persists for life. Animals undergoing a GVH reaction (GVH mice) display two distinctly different reactions towards tumor growth. During the early phase of the reaction, GVH mice possess a potentiated anti-tumor effect whereas, in the later stages, GVH mice experience a very high incidence of spontaneous lymphomas. We propose to use the GVH model to investigate both the mechanism of anti-tumor potentiation and also the factor(s) that promote the development of spontaneous lymphomas.

A. Immunopotentiation of anti-tumor resistance by the GVH reaction. In GVH mice the relationship will be determined between potentiation of anti tumor resistance and immune reactivity as measured by a variety of different immunological parameters, namely, humoral responses, mitogenic responses, specific and non-specific cell-mediated cytotoxicity, and natural killer cell activity, measured in vivo and/or in vitro. In addition, adoptive transfer of anti-tumor resistance from GVH to normal or immunodeficient hosts will be performed and the cell(s) responsible for the effect identified.

B. Spontaneous tumors arising in chronic GVH mice. We will investigate the cause(s) of the high incidence of spontaneous lymphomas in mice undergoing a chronic GVH reaction. Studies will include experiments on immune surveillance, induction of suppressor cells, activation of endogenous viruses, and the development of thymic dysplasia.

264. CHRONIC GRAFT-VERSUS-HOST DISEASE IN THE RAT RADIATION CHIMERA

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Chronic graft-versus-host disease (GVHD) is a frequent and often fatal sequela of human allogeneic bone marrow transplantation with features of scleroderma, Sjogren's syndrome and chronic active hepatitis. We have observed a very similar disease developing spontaneously in rat radiation chimeras. We have been able to induce chronic GVHD rapidly and consistently by two alternative methods: sublethal irradiation of chimeras followed by a booster bone marrow transplant and by adoptive transfer of chimera marrow and lymphocytes into third party irradiated recipients. Histology reveals dense dermal collagenosis, lymphoplasmacytic infiltrate and injury of the salivary and lacrimal gland ducts and chronic hepatitis with bile injury. Preliminary immunopathological studies suggest the presence of both allogeneic and organ specific antibodies.

This model has excellent potential for the study of the pathogenesis of chronic GVHD, a preclinical model for modes of therapy and prevention of chronic GVHD, and of probable value for studying some autoimmune disorders. We plan to thoroughly characterize the histopathology, distinguishing the disease from effects of aging, irradiation and infections. Immunopathological characterization will include definition of humoral and cellular components, antigen specificity of acid eluates of target organs and separation of the reactions against histocompatability antigens for reaction against organ specific antigens.

265. THE CENTRAL NERVOUS SYSTEM AS AN IMMUNOLOGICALLY PRIVILEGED SITE

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The objective of the research project is to determine if the immunologic privilege of the central nervous system will allow it to be more susceptible to adoptive immunotherapy than are neoplasms in other organs of the body. More specifically, we have been trying to document whether such immunotherapy using various immune populations can retard the growth of a rodent brain tumor.

The research plan that is employed has evolved over the past several years. Essentially, our protocol is based upon a comparison of the survival in control rats compared with those that have been periodically treated with injections of various types of immune cells directly into the animal's brain tumor cavity. More specifically, adult Fischer rats were injected stereotactically with 2.5 x 10 to the third power 9L tumor cells into the right cerebrum. With this treatment regimen untreated rats die from their brain tumor at approximately 21 days after injection. The treated animals were injected with lymph node cells that have been obtained from both normal animals and animals who have been specifically immunized against the tumor being used for the challenge.

The results of some of our experiments are as follows: the most effective cell population that we have discovered to date is that obtained from animals who themselves have a brain tumor and are subsequently challenged with irradiated tumor cells in incomplete Freund's adjuvant. Such cells can be made even more effective if they are cultured in vitro for 4 days in the presence of the irradiated tumor cells.

In the months to come, we will attempt to discover ways in which such immune lymphocytes can be made to reproduce in tissue culture, so that repetitive courses of immunotherapy can be carried out. The clinical implication of this work will be direct, because patients with clinically incurable glioblastomas can in fact be treated with a similar treatment protocol if it appears promising in this experimental rat model.

266. HEREDITARILY ATHYMIC-ASPLENIC MICE - TUMOR GROWTH

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This project is a continuation of our current studies on the heterotransplantation of human malignancies in immunodeficient animals, namely athymic (nude) and asplenic-athymic (lasat) mice. Chronic myelogenous leukemia (CML) cells with the Philadelphia chromosome from the cell line K-562 proliferate forming solid tumors (myelosarcomas) in nude mice associated with extensive metastatic proliferation when transplanted in lasat mice. The treatment of mice with xenoantisera to K-562 cells suppressed the growth of myelosarcomas. On the basis of these findings we propose to use the human cancer cell-animal tumor systems described above as models for the formation and subsequent growth of solid tumors, with the emphasis on the conditions of the host and/or the malignant cells required for local growth and metastatic proliferation.

The specific aims are: 1) To continue our current studies on the heterotransplantation of K-562 cells, 2) to continue to improve the treatment of myelosarcomas and metastases made of K-562 leukemia cells with specific antibody to K-562 cells, 3) to determine the mechanisms of action of the successful passive immunotherapy for myelosarcomas and metastatic proliferations of K-562 cells, 4) to develop drug-antibody conjugates for the treatment of mice bearing myelosarcomas and metastases, 5) to determine whether or not the natural cytotoxicity of nude mice is a major tumoricidal mechanism preventing the local growth and metastatic proliferation of myelosarcomas and other solid tumors of human origin mentioned in (6), 6) to develop new procedures for the heterotransplantation of human lung, breast and colorectal carcinomas, melanomas and the terminal blastic

crisis of a CML (these malignancies are the primary targets of concern because there is no reliable treatment; they are the most common types of cancer, very difficult to transplant in nude mice), and 7) to treat mice bearing human tumors with current and/or new therapeutic agents other than xenoantisera and antibody-drug conjugates. We propose various innovative approaches and novel ideas for the heterotransplantation of human malignancies.

267. CELLULAR IMMUNOTHERAPY OF MURINE TUMORS

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OBJECTIVE: Attempt to define the mechanisms of adoptive immunotherapy used alone or in combination with chemotherapy in the treatment of tumors of C57BL/6, BALB/c, and (BALB/cXC57BL/6)F1 mice. Emphasis will be on: development of additional models of adoptive therapy of established tumors, using not only cells immunized in vivo, but also cells significantly sensitized to TAA primarily or secondarily in vitro; determination of the effector cell(s) responsible for tumor therapy by isolating, purifying, and characterizing subpopulations of immune cells using sera directed against B-cells and subsets of T-cells and with the use of a fluorescent activated cell sorter; characterization and purification of the cell subpopulations which suppress the therapeutic efficacy of the effector cells; determination of which sensitization regimens in vivo and in vitro facilitate the generation of cells which enhance or suppress the therapeutic effect.

268. FUNCTIONAL AND MORPHOLOGICAL ANALYSIS OF MECHANISMS OF POTENTIAL SIGNIFICANCE FOR SPONTANEOUS METASTASIS

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OBJECTIVES: I. Experimental investigation of the effect of local X-ray treatment on systemic resistance against metastasis and the possibilities of preventing such an effect by lymphoid cell transfer using the mouse-tumor systems. II. Experimental investigation of possible correlations between morphological and functional changes of the local tissue reaction against tumor and changes of host tumor stage and degree of resistance against spontaneous metastasis.

APPROACH: I. Local irradiation is administered to various body regions and the effect on metastasis is recorded using syngeneic chemically induced tumors in C57BL/6J and CBA mice. Adoptive immunotherapy using various types and fractions of lymphoid cell populations is applied. II. Light-microscopic analysis of tumor-tissue sections using various morphological techniques for identification of monocytes-histiocytes and T/B lymphocytes. The investigations are carried out in various experimental situations with different rates of tumor progression and metastasis formation. Immune cell populations are at the same time isolated from central and peripheral lymphoid organs, blood and the tumor itself and their biological activity against tumor cells is assayed in vivo in neutralization tests.

269. IMMUNOTHERAPY OF METASTASES IN MICE WITH CYTOTOXIC SYNGENEIC T CELL LINES

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It is the objective of this study to determine if established lymphocyte cell lines derived from the syngeneic DBA/2 host can be utilized in the treatment of established visceral metastases. Initial experiments will establish the feasibility of initiating the regression of established metastasis in a minor histocompatability model. Ultimately, cytotoxic T lymphocytes obtained from the peripheral lymph node of a tumor-bearing DBA/2 mouse will be maintained in culture with conditioned medium and be reinjected intravenously into DBA/2 animals with established metastases. This procedure should provide a model for future attempts at in vitro sensitization and maintenance of human cytotoxic lymphocytes.

270. MECHANISMS AND THE RELATIVE INFLUENCE OF MACROPHAGES AND LYMPHOCYTES IN CELLULAR IMMUNOTHERAPY

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OBJECTIVE: Lymphoid cells from immunized donors injected into tumor-bearing mice prolong the survival time of these hosts. It has been suggested that this antitumor effect might result from some form of cooperation of the transferred lymphocytes and the host macrophages. Within this context it seems reasonable to expect that the antitumor effect might be enhanced by adding macrophage-like cells to the injected lymphocytes.

APPROACH: To test this hypothesis histiocytic leukemia cells were used as a source of macrophages. DBA/2 mice were injected with SL2 cells and with immune (versus SL2) DBA/2 spleen cells and/or BALB/c-derived J774A histiocytic leukemia cells.

PROGRESS: The treatment with immune spleen cells resulted in a prolongation of the survival time of the tumor-bearing mice. Addition of J774A cells to the immune spleen cells resulted in a consistent enhancement of the antitumor effect. Addition of BALB/c-derived spleen cells, MOPC 195 cells or lysed J774A cells did not result in an enhancement of the antitumor effect. J774A cells by themselves were ineffective in prolonging the survival time of SL2-bearing DBA/2 mice.

Preliminary studies show that the antitumor activity of immune spleen cells can be enhanced in vitro by co-culture with J774A cells. This means that in vivo J774A cells also might stimulate the antitumor activity of the immune spleen cells. It is also possible that the immune spleen cells arm and activate the J774A cells in vivo. Finally, the antitumor effect might be due to a mutual stimulation of both macrophage-like cells and immune lymphocytes. The result would be that both cell types kill the tumor target cells.

271. CELLULAR IMMUNE RESPONSES TO SYNGENEIC TUMORS

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The tumor we are using in this study is the Sarcoma I (Sa I) tumor which is maintained in the ascites form in syngeneic A/J (H-2a) mice by weekly serial transplantation. This tumor is also maintained in tissue culture by our laboratory. Injections of 10 to the third power to 10 to the fourth power Sa I cells kill 60-80% of A/J mice in 30-50 days. Immunity to this challenge dose can be induced in A/J mice by pretreatment with irradiated Sa I cells. We have recently demonstrated that splenectomy performed three weeks prior to tumor challenge reduces the mortality from 60-80% to 20-40%. At the time of tumor challenge the effect of splenectomy can be reversed by adoptive transfer of spleen cells that do not adhere to Sephadex G-10. These findings indicate that the spleen contains lymphocytes which regulate the immune response against syngeneic tumors. The specific aims of this research project are to: (1) Identify the spleen cell subpopulation of lymphocytes involved in the regulation of the immune response against syngeneic tumors; (2) produce continuous tissue culture cell lines of T lymphocytes with cytotoxic activities against the Sa I tumor; and (3) extend these studies to other tumor-host systems. The overall goal of this research project is to gain a better understanding of the immunological reactions between the host and the tumor in terms of the cells (B cells, T cell, null cells, and macrophages) involved in the immunological rejection and enhancement of tumor growth.

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272. GENERATION OF CYTOTOXIC LYMPHOCYTES AGAINST LYMPHORETICULAR MALIGNANCIES

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The objective of the proposed research is to generate large numbers of cytotoxic lymphocytes in vitro, capable of lysing autologous human leukemia and lymphoma cells, that could ultimately be used immunotherapeutically in patients following tumor cytoreduction. Attempts will be made to generate cytotoxic T lymphocytes (CTLs) by culturing patients' T cells in vitro with: 1. Autologous malignant cells and allogeneic normal cells (in 'three-cell' protocols), 2. allogeneic malignant cells of the same type as the patients's, 3. allogeneic normal lymphocytes pooled from 20 individuals, or 4. concanavalin A. CTLs generated by these methods will be restimulated or grown in media containing T cell growth factor to generate large numbers of CTLs. These CTLs will be used for studies aimed at determining whether malignant cells from different patients share common target antigens. To determine whether in vitro generated CTLs can inhibit leukemia development or progression in vivo, mouse CTLs that lyse syngeneic leukemia cells will be generated in vitro by similar protocols and will be tested in vivo in immunoprophylaxis and immunotherapy protocols.

273. ANTI-TUMOR ACTIVITY OF TUMOR-BEARER LYMPHOID CELLS

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The effectiveness of adoptive cellular immunotherapy depends on the ability of the infused cells to survive in the recipient host and to implement a potent anti-tumor response. An ideal source of such lymphoid cells is the tumor-bearer's own cells since they are not only histocompatible but also primed to tumor antigens. Our goal is to generate maximal levels of anti-tumor cytotoxicity in tumor-bearer lymphoid cells and to evaluate the therapeutic value of such cells. To achieve this goal we will first identify; 1) The cellular requirements for the generation and expression of anti-tumor cytotoxicity in tumor-bearer spleen cells upon in vitro activation with stimulator tumor cells; 2) the nature of the suppressor cell(s) among tumor-bearer lymphoid cells as well as their mode of action in inhibiting the anti-tumor activity in tumor-bearer spleen cells that are cultured in the presence or absence of stimulator tumor cells. This information will guide us in the search for methods to selectively stimulate potentially cytotoxic cells and/or selectively remove suppressor cells resulting in the generation of augmented levels of anti-tumor cytotoxicity upon in vitro activation. We will evaluate the homing patterns of activated tumor-bearer lymphoid cells and determine whether changes in the tendency of infused cells to home to lymph nodes draining the site of tumor injection can be used to screen lymphoid cells for their potential effectiveness in transferring systemic anti-tumor activity. The effectiveness of infused activated tumor-bearer lymphoid cells in transferring systemic anti-tumor activity will be determined in relation to the tumor burden that they can cope with. We will employ adoptive immunotherapy as an adjunct to cytostatic therapy for specific eradication of residual tumor cells. We will evaluate the nature of immunity in mice cured of tumor following immunotherapy to determine which anti-tumor responses are altered and augmented in comparison with these responses in mice with progressively growing tumors.

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274. GENERATION OF EFFECTOR CELLS FOR TUMOR IMMUNOTHERAPY

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The objectives of the current proposal are to characterize the in vitro culture system by which specifically sensitized lymphocytes are generated by cocultivation with murine mammary tumor cells, to utilize it to ascertain the basic mechanisms underlying the in vivo immune responses to mammary tumors, and to determine optimal conditions for obtaining successful therapeutic effects when lymphocytes sensitized in vitro are transferred into tumor bearing mice.

In the initial studies, the optimal conditions for in vitro generation of cytotoxic effector activity against syngeneic mammary tumor cells were determined. The cytotoxic effector activity obtained under these conditions was mediated by T-cells and specific against mammary tumor cells of the same H-2 haplotype, although less though significant cross-reactivity was observed against allogeneic mammary tumor cells.

In the coming year, studies will be performed to investigate the immunotherapeutic effects of the immune effector cells by determining the fate of these cells after transfer into tumor bearing mice.

275. IMMUNOLOGIC INHIBITION OF TUMOR GROWTH

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We have a reproducible model of adoptive tumor immunity in which lymphoid cell subpopulations from tumor-bearing mice may be tested for their efficacy in the inhibition of tumor growth, and those tumor cells which survive this immunologic attack may be tested for protective alterations in their tumor specific transplantation antigens. Experimental procedures are proposed to further refine and define this assay system as to the composition of the responding cellular elements and their identity. The proposal will attempt to identify the lymphoid cell or cells which are able to inhibit tumor growth in vivo and to identify what, if any, lymphoid cell subpopulations are present which may regulate this protective response. When these experiments are completed the defined adoptive transfer model will be used to test the hypothesis of protective tumor cell antigenic adaptation. Experiments will be performed to test whether such an antigenic loss or change occurs and whether it is temporary or inheritable. Further experiments will be designed to assay the antigenic character of tumor cells which metastasize and determine whether certain antigenic changes are associated with metastasis to specific anatomic sites. Additionally, this proposal will attempt to study the relationship of tumor rejection antigens identified in tumor-free immunized animals to those in tumor-bearing animals. This proposal will attempt to study the adaptive response of the host immunologic system and the tumor cells to each other during the natural history of tumor-bearing. Knowledge of this adaptive interplay is fundamental to the design and understanding of future immunotherapeutic and combined immunotherapeutic and chemotherapeutic treatment of tumors.

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3. TRANSFER FACTOR AND IMMUNE RNA

276. PASSIVE TRANSFER OF CELL-MEDIATED IMMUNITY BY TRANSFER FACTOR

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OBJECTIVES: (1) Determine if bovine transfer factor can promote a beneficial response in mice with an immune deficiency such as that observed after radiation damage or in certain disease states. (2) Determine the effect of irradiation on the beneficial response elicited by transfer factor in diseased mice. (3) Investigate the mechanism by which transfer factor promotes beneficial responses in mice.

APPROACH: (1) Mice will be treated with transfer factor before and after a disease state is induced in the mice. The amount of transfer factor and the time at which it is given will be varied to optimize the response in the mice. (2) Mice will receive sublethal doses of ionizing radiation before and after treatment with an optimum dose of transfer factor. The disease will then be induced and the effect of the various treatments will be assessed by the survival times. (3) Lymphocytes from mice treated with transfer factor will be assayed in vitro to determine the subpopulation which is responsible for the response and the mechanism by which the cells act against the disease.

277. SEQUENCING OF TRANSFER FACTOR

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This is part of a broader project. A summary of this subproject is not available.

278. BIOCHEMICAL APPROACH TO THE SEPARATION AND PURIFICATION OF TRANSFER FACTOR

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This is part of a broader project. A summary of this subproject is not available.

279. HUMAN TRANSFER FACTOR TO MOUSE TRANSFER SYSTEM

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This is part of a broader project. A summary of this subproject is not available.

280. HUMAN TRANSFER FACTOR IN MICE TREATED WITH THYMOPOIETIN

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This is part of a broader project. A summary of this subproject is not available.

281. EFFECT OF TRANSFER FACTOR ON GROWTH OF SV40 TUMORS (MICE)

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This is part of a broader project. A summary of this subproject is not available.

282. MURINE TRANSFER FACTOR TO MOUSE TRANSFER SYSTEM

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This is part of a broader project. A summary of this subproject is not available.

283. EFFECTS OF RABBIT ANTIBODY TO HUMAN TRANSFER FACTOR

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This is part of a broader project. A summary of this subproject is not available.

284. IN VITRO ASSAY OF TRANSFER FACTOR ACTIVITY (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

285. EFFECTIVENESS OF ANTI-VARICELLA TRANSFER FACTOR IN NON-IMMUNE LEUKEMIC CHILD

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This is part of a broader project. A summary of this subproject is not available.

286. TUMOR IMMUNOTHERAPY WITH 'IMMUNE' RNA

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Our objective is to develop a regimen for immunotherapy of tumors using RNA-rich extracts from syngeneic or xenogeneic animals immunized to the tumor. The rationale of immunotherapy is to convert syngeneic nonsensitive peritoneal exudate cells (NS-PEC) with 'tumor-immune' RNA extracts so that they become immunologically reactive to the tumor specific antigen (TSAg), which is then added to specifically stimulate the converted cells. Our proposal is based on experiments with a transplantable diethyl-nitrosamine-induced hepatoma in strain-2 guinea pigs. We already found that when syngeneic NS-PEC, syngeneic or xenogeneic 'tumor immune' RNA, and TSAg are injected s.c. 5-days after the injection of a uniformly lethal dose of line-10 tumor cells, complete tumor regression of the intradermally growing tumor was observed in all 24 strain-2 guinea pigs treated. Omission of either the NS-PEC, RNA or TSAg from this regimen was ineffective in tumor abrogation. Since all animals were treated after metatases are frequently known to occur, the long-term tumor-free survival of these animals suggested that the 'RNA therapy regimen' may induce a systemic immunity against the tumor. Our specific aims now are as follows: 1) To determine the optimal conditions for abrogation of tumors established by i.d. injection of uniformly lethal doses of diethylnitrosamine-induced hepatoma cells; 2) to assess tumor immunity in guinea pigs cured of tumor; 3) to assess the effect of local therapy on metastases using tumor cells injected at a second site or multiple sites as a model; 4) to compare the effectiveness of 'tumor immune' RNA by reciprocal experiments with line-10 and another line; 5) to explore RNA therapy in combination with other methods, e.g., anti-fibrinopeptide E, BCG, or chemotherapy; 6) to extend the immunotherapy model to other tumors and other species; 7) to isolate and characterize the active RNA. The methods involve the preparation of tumor immune RNA rich extracts from a variety of animals, the preparation of TSAg from a variety of tumors, histological studies of treated sites, and the maintenance of tumor lines. This immunotherapy model appears to be better than BCG for this tumor. Its requirements and limitations need to be fully characterized.

287. IMMUNOTHERAPY OF INTRACRANIAL TUMORS IN MICE WITH THE USE OF IMMUNE RNA EXTRACTS

Labadie, E. L., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Neurology Section, *3601 S. 6th St., Tucson, Arizona*, 85723, United States of America

PROGRESS REPORT: As expressed in our previous progress report, after having studied over 1,000 mice using immune RNA versus anti-tumor immune spleen cells, it was found that extracts of immune RNA were totally unsuccessful in attenuating intracranial live tumor implants; however, even in small ratios of two donor spleen cells given to one recipient statistical evidence of immunological transfer was shown. At that point the study was terminated since it was clearly evident that immune RNA extract in BALB/c mice either presents serious technical difficulties or it does not produce therapeutic effects.

During those experiments several unexpected incidents occurred in which we inadvertently provoked 'tumor enhancement' (or immunological paralysis). We then decided to use a model of experimental allergic encephalomyelitis in rats and tried to replicate that 'immunological paralysis' and 'immunological enhancement' since both effects have important clinical implications.

Up to this date our studies of immunotherapy of intracranial tumors have been presented at the North Central Mexico and Southwestern U.S.A. Neurology/Neurosurgery/Psychiatry Meeting in Tucson, Arizona, in October 1979. The results on the EAE rat model have been submitted for merit review and some of these will be prepared for publication shortly.

288. TRANSFER OF TUMOR IMMUNITY WITH IMMUNE-RNA

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The present study is concerned with the application of the transfer of cellular immunity by immune-RNA (I-RNA) to the immunotherapy of tumors. In animal studies utilizing a syngeneic mouse tumor-host system, the duration of tumor specific immunity transferred by normal syngeneic spleen cells incubated in vitro with I-RNA capable of transferring tumor-specific immunity will be looked for and the efficacy of tumor specific immunity transferred by I-RNA will be studied in an immunotherapy protocol which involves the prevention of metastases following excision of a primary tumor. Comparison will be made of in vitro correlates of tumor specific immunity and the results of immunotherapy in vivo. The mechanism of I-RNA mediated transfer of tumor immunity will also be investigated in several in vitro systems.

289. MODULATION OF IMMUNE RESPONSES BY RNA

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PROJECT OBJECTIVES: 1) We want to test the hypothesis that the immunologic deficiency of human and mouse myeloma is due to B lymphocyte disfunction induced by tumor RNA, among other factors. 2) The genetically determined unresponsiveness of C3H/HeJ mice to LPS may be corrected by xenogeneic RNA.

PLAN: Peripheral blood or splenic lymphocytes will be cultured in the presence of RNA isolated from lymphocytes of patients with lymphoproliferative disorders. The role of xenogeneic RNA as amplifier of immune response of C3H/HeJ mice will be similarly studied. Results of our investigations indicate that: 1) RNA from patients with lymphoproliferative disorders converts normal B cell SIg to the SIg idiotype synthesized by the malignant cell, and 2) xenogeneic RNA corrects the genetically transmitted unresponsiveness of HeJ mice to LPS.

CLINICAL RELATIONSHIP: The area of our research is highly relevant to the understanding of some of the mechanisms responsible for the immunodeficiency of patients with malignant immunoproliferative disorders. We have recently developed a test for the detection of the cystic fibrosis gene in man.

D. EXPERIMENTAL IMMUNOTHERAPY OF SPECIFIC CANCERS

1. EXPERIMENTAL IMMUNOTHERAPY OF LEUKEMIAS, LYMPHOMAS, AND SARCOMAS

290. IMMUNOTHERAPY OF TRANSPLANTED LEUKEMIA BY MONOCLONAL ANTIBODY

Hammerling, U. G., Sloan Kettering Inst. for Cancer Research, Dept. of Immunochemistry, *410 E. 68th St., New York, New York*, 10021, United States of America

Monoclonal antibodies to cell surface antigens will be tested for their ability to suppress growth of transplanted leukemia in mice. We propose to use a) model systems in which tumors are transplanted into mouse strains congenic to the strains of tumor origin (e.g., strains congenic at the Thy-1 or Ly loci), b) tumor cells with known virus-controlled surface antigens (e.g., gp70 determinants) and tumor associated antigens (e.g., X.1), transplanted into syngeneic hosts. Cell lines secreting monoclonal antibodies to each of the antigens named above have been produced by the Kohler and Milstein procedure. In several cases different immunoglobulin subclasses are available with specificity for the same antigen. These antibodies form the basis for the planned investigation in which we will ask: 1) Whether administration of antibody can alter the growth pattern of established tumors, 2) what dose, route of administration, or immunoglobulin subclass would optimally induce remission, 3) whether utilization of antibody as a vector for transport of cytotoxic drugs or radionuclides to the tumor sites can improve the efficacy of immunotherapy, 4) whether monoclonal antibody to selected types of MuLV given in early life can delay the onset of leukemia in AKR mice.

291. IMMUNOTHERAPY OF MURINE LEUKEMIA

Collins, J., Duke University, School of Medicine, Dept. of Surgery, *Box 3711, Durham, North Carolina*, 27710, United States of America

This is part of a broader project. A summary of this subproject is not available.

292. ANTIGENIC ALTERATIONS OF TUMOR CELLS BY PHARMACOLOGICAL MEANS

Nicolin, A. N., Franco, P., Marelli, O., Veronesi, F., Testorelli, C., Sarra, F., Peroni, D., PFCCN, *Via Vanvitelli 32, c/o Inst. of Pharmacology, 20129 Milan, Italy*

OBJECTIVE: To induce, by pharmacological means, increased antigenicity in tumor cells to be exploited for immunoprophylaxis and immunotherapy of experimental tumors.

APPROACH: The compound 5-(3, 3-dimethyl-1-triazeno) imidazole-4-carboxamide (DTIC) has been used to alter the antigenicity of murine lymphoma cells by in vivo and in vitro treatments. Host immune response, antigenic features and relevant biological characteristics of DTIC-treated cells are studied.

PROGRESS: Increased antigenicity has been obtained in a variety of murine tumors following in vivo treatments and similar results have been preliminarily obtained by in vitro treatments. Host immune responses to drug-induced antigens have been studied in some detail. The vaccinogenic properties of drug-treated cells have been successfully exploited for immunoprophylactic studies and, in association with effective chemotherapy, for immunotherapeutic trials in laboratory animals. The nature of the drug-induced antigens as well as possible surface alterations of drug-treated cells is under study. No differences have been noticed in the quantitative expression of normal antigens whereas increased Con A agglutinability and increased susceptibility to T cell mitogens have been found in DTIC-treated cells.

293. IMMUNOTHERAPY IN OUTBRED ANIMAL LYMPHOMA AND LEUKEMIAS

Essex, M. E., Harvard University, Cambridge Campus, School of Public Health, Dept. of Microbiology, *Cambridge, Massachusetts*, 02138, United States of America

Cats with chemotherapy-induced complete remission of thymic lymphoma will be randomly assigned to no further therapy or treatment with antiserum against feline oncornavirus associated cell membrane antigen (FOCMA). Animals entered in the study will be monitored clinically and immunologically with various in vitro and in vivo assays including FOCMA antibody titers and blast transformation with Con A, lipopolysaccharide, FOCMA antigen, and FOCMA-specific antibody-dependent cell-mediated cytotoxicity. Cats with ALL will be treated with immunoglobulins prepared from anti-FOCMA or control sera and administered with and without fresh normal serum to try to determine whether other serum components influence the therapeutic activity of anti-FOCMA antibodies, if appropriate.

294. A FELINE LYMPHOMA MODEL FOR CHEMO-IMMUNOTHERAPY STUDIES (CAT)

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This is part of a broader project. A summary of this subproject is not available.

295. THE IMMUNOTHERAPY OF ANIMAL AND HUMAN SARCOMAS

Rosenberg, S. A., Strausser, J., Lotze, M., Almaraz, R., Eberlein, T., Rosenstein, M., Brown, J., Yron, I., Schwarz, S., Wexler, H., Spiess, P., Hyatt, C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Surgery Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The summary for this project is under entry no. 42.

296. THERAPY OF CANINE OSTEOSARCOMA AND CANINE MAMMARY CARCINOMA

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PURPOSE: The purpose of the research is to study methods of therapy in two canine malignant tumors which are of considerable comparative interest to malignant tumors in man, namely osteosarcoma and mammary carcinoma. This will be done by randomized trials. A. Osteosarcoma: To determine if irradiation with hypoxic cell sensitizer is more effective for local tumor control and preservation of limb function than irradiation alone. To determine if lung irradiation with or without a hypoxic cell sensitizer will: (a) Increase the period of time to radiographic evidence of metastasis, (b) increase time of survival. To continue studies on pharmacokinetic, tumor penetration and toxicological properties of newly developed hypoxic cell sensitizing drugs in normal dogs. B. Mammary Carcinoma: The main aim is to determine if immunotherapy following mastectomy for invasive tubular adenocarcinoma or solid carcinoma is better than mastectomy alone. The secondary aim is to determine if live BCG vaccine has a therapeutic effect different from that of inactivated C. parvum vaccine.

APPROACH: The osteosarcoma trial will be conducted using either a linear accelerator or Cobalt machine and a total of 4000R (8 fractions) will be given to the primary tumor. Dogs with mammary carcinoma will be given either C. parvum or BCG post-mastectomy. The World Health Organization Collaborating Center for 'The study of the biological behavior and therapy of neoplasms in domestic animals' has been designated to be located at the Department of Veterinary Clinical Medicine in the University of Cambridge under the direction of Dr. L.N. Owen. Details of the trials are available on request and active collaboration is welcomed. A minimum of 200 days is needed for each trial.

297. IMMUNOPROPHYLAXIS OF BOVINE LYMPHOSARCOMA

Kenyon, S. J., University of Pennsylvania, School of Veterinary Medicine, Dept. of Clinical Studies, *39th & Pine, Philadelphia, Pennsylvania,* 19104, United States of America

The University of Pennsylvania, School of Veterinary Medicine, owns a herd of cattle (the BF herd) with a high incidence of naturally occurring lymphosarcoma (65 cases in 17 years). The animals in this herd are well characterized in terms of pedigree, hematologic parameters, bovine leukemia virus (BLV) infection, and lymphosarcoma risk (as determined by pedigree analysis). Since BCG has been found to influence chemical and viral tumorigenesis in inbred strains of laboratory animals we propose to evaluate the protective effect of this agent against a naturally occurring tumor. It is proposed to vaccinate 31 animals of high lymphosarcoma risk by intravenous administration of BCG cell walls in oil droplet. Thirty-one age-matched cattle of equivalent lymphosarcoma risk, bred from the same foundation stock and kept in the herd under the same management conditions will serve as uninoculated controls. It is expected that 6 animals in the control group will develop lymphosarcoma within the 3-year period of the initial contract.

Studies of the effect of BCG vaccination on lymphocyte subpopulations in these cattle as opposed to the controls will be carried out. A benign lymphoproliferative condition, persistent lymphocytosis (PL), frequently accompannies BLV infection. The increase in absolute numbers of lymphocytes in the peripheral blood of these cattle is due predominantly to an increase in the numbers of B lymphocytes, of which two sub-populations have been recognized. One population is BLV-infected and the other is an apparently immunologically reactive population, which spontaneously incorporates 3H-thymidine after 3 days in culture. This activity is inhibited by in vitro culture in the presence of serum from any BLV-infected cow. An expanded population of non-B lymphocytes is also present in these cattle and spontaneously incorporates 3H-thymidine after 7 days in culture. Serum from some cows with lymphosarcoma is capable of inhibiting this activity as well as PHA-induced lymphocyte blastogenesis by lymphocytes from BLV-free cattle. At the time of tumor development marked changes in the number and activities of these peripheral blood lymphocyte populations have been observed. (Text Abridged.)

298. IMMUNOTHERAPY OF MURINE SOLID TUMORS

Iglehart, D. J., Duke University, School of Medicine, Dept. of Surgery, *Box 3711, Durham, North Carolina,* 27710, United States of America

This is part of a broader project. A summary of this subproject is not available.

2. EXPERIMENTAL IMMUNOTHERAPY OF OTHER SPECIFIC CANCERS

299. TREATMENT AND/OR PREVENTION OF LUNG CANCER BY IMMUNOTHERAPY USING A SUCCESSFUL EXPERIMENTAL CENTRAL TYPE LUNG CANCER INDUCED IN DOGS

Hayata, Y., Oho, K., Taira, O., Ogawa, I., Tokyo Medical College Hospital, Dept. of Surgery, *6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160, Tokyo, Japan*

In order to evaluate Nocardia rubra cell wall skeleton (N-CWS) immunotherapy in terms of treatment of early stage lung cancer and also to estimate whether it can prevent the development of cancer at a precancerous stage, we are now administering N-CWS in a successful and reproduced experimental series in which squamous cell carcinoma can be induced in dogs.

In each group, after squamous cell metaplasia, carcinoma in situ or invasive carcinoma has been induced in the dogs (beagles and mongrels) by submucosal injections of the carcinogen, the PPD skin test is performed, followed by injection of 200mg of N-CWS intradermally or intratumorally. Thereafter, while continuing the weekly administration of the carcinogens, 100mg of N-CWS is administered at two week intervals. Changes of the bronchial epithelium are examined cytologically, histologically and bronchofiberscopically and various immunoparameters are also monitored throughout the experiment.

300. IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY OF LUNG CANCER

Zwilling, B. S., Ohio State University, Columbus Campus, School of Biological Sciences, Dept. of Microbiology, *190 N. Oval Dr., Columbus, Ohio,* 43210, United States of America

This study is designed to determine the potential of BCG preparations to prevent the development of primary bronchogenic carcinoma in Syrian golden hamsters. Results indicate that BCG cell walls when administered after carcinogen exposure (BP-Fe2O3) significantly reduced lung tumor incidence from 75% in control animals to 50% in treated animals.

Additional studies concerning the effect of BCG on alveolar macrophage tumoricidal capacity have shown that three distinct activation states can be identified. Macrophages from BCG immune-rechallenged hamsters are capable of destroying tumor cells while macrophages from BCG immune or normal hamsters do not. The tumoricidal capacity has been correlated to the response to chemotactic stimuli. Other functional parameters are being assessed to more closely identify characteristics of normal, activated-nontumoricidal and tumoricidal macrophages.

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301. REGIONAL PERBRONCHIAL CHEMOIMMUNOTHERAPY OF BRONCHOGENIC CANCER

Brody, A. W., Chaperon, E. A., Albano, W. A., Creighton University, School of Medicine, Dept. of Medicine, *629 N. 27th St., Omaha, Nebraska,* 68131, United States of America

We have been formulating a model permitting local regional perbronchial chemotherapy and immunotherapy of a developing cancer in the bronchus and lung of rats, that permits statistical comparisons of different chemotherapeutic agents, and comparisons of each agent alone, or of each group of agents, against that agent(s) plus immunotherapy. The model consists first of placement of a sufficient bolus of suspended viable cancer cells to dependably reproduce a cancer into a selected bronchus of each rat, and 3 or 4 days later (and thereafter at appropriate intervals) placing a bolus of chemo- and/or immunotherapeutic

agent(s) into the same segment. While we are now using Walker carcinosarcoma in Sprague-Dawley outbred rats for the development of techniques, we intend to continue with an allogenic bronchogenic carcinoma in an inbred line. The same method was used (and will be) to establish dose-toxicity curves for the chemotherapeutic agent(s) used via this route (intrabronchial, intrapulmonary) in order to determine the largest dose for safe use. A second model permits repeated interval perbronchial retreatments conveniently.

Methods and techniques for corresponding regional perbronchial therapy via bronchoscopy will also be formulated in dogs for eventual use in human patients with bronchogenic cancer.

302. THERAPY OF CANINE OSTEOSARCOMA AND CANINE MAMMARY CARCINOMA

Owen, L. N., White, R., University of Cambridge, Dept. of Veterinary Clinical Medicine, *Hills Rd., Cambridge CB2 1QT, England, United Kingdom*

The summary for this project is under entry no. 296.

303. INTRALESIONAL IMMUNOTHERAPY TREATMENT OF BREAST CARCINOMA

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OBJECTIVE: Evaluate in a controlled prospective clinical study the effect of intralesional injection of immunoadjuvant(s) on canine breast carcinomas. Dogs clinically free of detectable metastatic disease will be randomly assigned to intralesional placebo prior to surgery or intralesional immunotherapy prior to surgery. The immunoadjuvant materials will be provided by the National Cancer Institute. Tumor regression, tumor recurrence, disease-free interval, and survival data from the two groups will be compared. Selected assays of humoral and cellular immunity will be performed and results correlated with clinical course. It is estimated that 100 dogs per year will be entered in the study.

304. IMMUNOTHERAPY OF MAMMARY TUMORS

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The overall objective of this study is to develop and use the mouse mammary tumor system as an experimental model where immunotherapeutic procedures may be evaluated. Treatment procedures will be compared with respect to their ability to induce, enlarge, and maintain the host's immune resistance factors. The planned research aims to develop the mouse mammary tumor system for exploring and testing procedures of immunotherapy. The experiments are designed to reveal fundamental aspects of tumor immunogenicity and of anti-tumor immunity, and to indicate effective and hazardous therapeutic procedures. The objectives are mainly four: (1) To investigate the effects on host resistance caused by immunotherapy in autochthonous and in syngeneic hosts at different stages of tumor development and growth, and at different times relative to surgery, radiotherapy, and chemotherapy. (2) To investigate conditions and procedures which may increase or reduce the risk of specific depression of host resistance by excess free tumor antigen or blocking factors. (3) To study the frequency of occurrence and the immunogenic characteristics of mammary tumors arising spontaneously in mice free of exogenous MTV-S (milk factor) virus, such as in C3Hf/He, C3Hf/Ki, C3Hf/Bi, C3Hf/An, C3Hf/St and C3Hf/StW sublines, some of which are also presumed to be free of endogenous MTV-L (germ-line-transmitted) virus such as C3Hf/Ki, C3Hf/Bi, and C3Hf/StW. (4) To investigate the nature of the tumor growth stimulation effect associated with some C3H/H3 and C3Hf/He mammary tumors, and associated with apparently all C3H/Ki and C3Hf/Ki mammary tumors.

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305. CELL-MEDIATED IMMUNITY IN MOUSE MAMMARY TUMOR MODELS

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Three different lines of BALB/c mice are being studied in connection with their incidence of spontaneous mammary tumors (SMT), their expression of mouse mammary tumor virus (MMTV) particles and/or antigens, and their immune response. These lines are: 1) BALB/cCrgl which has a low incidence of SMT, is devoid of intact MMTV but expresses some viral antigens and an in vitro immune response to these antigens as well as to tumor antigens of their syngeneic chemically induced mammary tumors; 2) BALB/cfC3H which has a high incidence of SMT and in which MMTV particles can be detected and 3) hereditarily asplenic BALB/c mice heterozygous for the nude gene, which have a high incidence of SMT (45-50%) with a mean age of appearance of 10 months. The proposed research intends to identify, characterize and isolate the immunologically relevent cells known to respond to either viral or tumor antigens and to use them in adoptive transfer experiments. Studies will include the nature of cellular and humoral in vitro reactions and the possible role of suppressor cells. This immunotherapeutic approach aims at achieving protection of mice with a high risk of developing SMT, thus providing insight into the mechanism(s) operative in tumorigenesis.

306. IMMUNOTHERAPY OF MAMMARY TUMORS

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The proposed research aims to use the mouse mammary system as an experimental model for cancer immunotherapy, to reveal fundamental aspects of antitumor immunity, and to indicate effective and hazardous immunotherapeutic procedures. Areas of investigation are primarily four: 1. To study the nature of the local immune reaction against mammary carcinoma MC1 in subcutaneous and intra-mammary implantation sites, and in the lungs after induced metastatic dissemination. The tests will use monoclonal antibodies against cell surface markers and peroxidase staining to identify the various effector cell types involved in the immune rejection response. 2. To study the supportive and the negative effects which may result when immunological treatments are given at different stages relative to primary tumor cure and secondary tumor development. The tests will use bacterial immunopotentiators and killed tumor cells. 3. To study the effects on tumor growth and regression when humoral and cellular tumor host factors are passively transferred to syngeneic athymic and normal tumor hosts. The tests will use whole blood and cells isolated from the primary stromal reaction against implants of MC1. 4. To search for a more relevant experimental mammary cancer system by determining the viral or nonviral etiology of tumors from different C3H sublines.

307. IMMUNOTHERAPY OF EXPERIMENTAL BRAIN TUMORS

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Because of the ways in which CNS neoplasia is unique, it appeared to us that intra-tumor adoptive immunotherapy offered the most promise. In order to determine which population of immune cells would be most effective in retarding tumor growth, we carried out a series of Winn assays. In our first set of experiments, we determined that peritoneal exudate cells from animals that had been previously immunized against the specific tumor cell line could prevent the subcutaneous growth of this brain neoplasm. We next determined that such a cell population would in fact be as effective in retarding brain tumor growth when injected intracerebrally either alone or combined with systemic brain tumor chemotherapy.

Since it would appear difficult to conceive of using peritoneal exudate cells in any form in human brain tumor immunotherapy, we next evaluated the effectiveness of lymph node cells when using adoptive immunotherapy. The results of these experiments indicated that the most effective lymph node population was that which was obtained from previously immunized rats, and in which the lymph node cells were not passed over

nylon wool columns. Once again, when this cell population was injected intracerebrally, we achieved the prolongation in survival in animals harboring a 9L brain tumor. In our most recent experiments, we concluded that irradiated whole tumor cells were a better mode of antigen presentation compared to either gluteraldehyde-treated tumor cells or a KCl extract of the tumor cells. In addition, other experiments indicated that peripheral lymph node cells obtained from animals who themselves have brain tumors are at least as effective as donor cells taken from animals who do not themselves harbor a brain tumor.

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308. CANCER EYE (IMMUNOTHERAPY AND IMMUNOPROPHYLAXIS)

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OBJECTIVE: Evaluate immunotherapeutic agents in the prevention of bovine squamous cell carcinoma.

APPROACH: Using tissue culture techniques, explore the relationships and interactions between BCG cell wall vaccine and malignant cells in culture in an attempt to elucidate the mechanism by which the vaccine is effective. If such a mechanism if found, maneuvers to increase the efficacy of cell wall therapy by manipulation of this mechanism can be attempted.

PROGRESS: Procurement: This contract was designed to determine the efficacy of cancer immunotherapeutic agents and to contribute to the development of human clinical protocols in the treatment of malignant disease. Eighty percent of the required tumor-bearing cows have been obtained and treated with various agents. Additionally, 10 tumor-bearing horses (100% of contract requirements) have also received immunotherapy. Documentation of clinical and experimental procedures will follow for the remaining contract period. Ten additional cows will be obtained for treatment with therapeutic agents to be determined by the National Cancer Institute. Immunoprophylaxis: This contract was designed to determine the efficacy of BCG cell wall vaccine in the interruption of the transformative process of tumors from benign to malignant. The first year of this contract calls for obtaining 240 lesion-bearing animals and initiating treatment in experimental groups. To date, 95 animals have been obtained, biopsied, tagged, and identified and placed into proper experimental groups.

309. CLINICAL AND BIOLOGICAL MANIFESTATIONS OF IMMUNOTHERAPY IN CANCER EYE OF CATTLE

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OBJECTIVE: Evaluate the manifestations of immunotherapy in cattle afflicted with bovine ocular squamous cell carcinoma (cancer eye). Establish squamous cell neoplastic tissue in culture. Attempt to isolate an etiologic agent. Identify tumor-associated antigens serologically and by immuno-electron microscopy.

APPROACH: Tumor bearing animals will undergo injection either by the intramuscular route, into the bulbar conjunctiva or directly into the tumor. The separate inoculum will consist of lymphocytes from sensitized rabbits or calves, immune serum from the same animals and a phenol extract of lysed squamous cells from an active carcinoma. Serum samples will be collected routinely to be assayed by immuno-electron microscopy, immuno-fluorescence, virologic and serologic procedures. Clinical evaluation of tumors will be accomplished weekly and a permanent record will be prepared on film.

PROGRESS: Six cows each bearing histopathologically defined squamous cell carcinomas (BOSCC) were divided into 2 groups of 3 and treated individually by immunotherapy with 2 different modals. Three cows were treated with neuraminidase treated cells injected into the tumor mass at 3-week intervals for 7 months; and three cows were treated with Bacillus Calmette-Guerin (BCG) by injection of this adjuvant into the base of the tumor mass. BCG was injected during two treatment periods, with a 6-7 weeks rest separating the periods. Biopsy tissues were collected at the completion of the experiment and submitted for histopathologic examination. Blood samples were collected monthly to monitor the blastogenic response of bovine lymphocytes to pokeweed mitogen and BOSCC antigens. All tumors injected with neuraminidase treated cells grew progressively without significant microscopic changes in the malignant tissue. One tumor was treated with liquid nitrous oxide to arrest tumor growth by freezing. Although the exophetic tumor mass was reduced by necrosis, the malignant tissue invaded the orbital sinuses. One small (6 mm) tumor treated with BCG regressed after the first treatment series. This tumor was not treated during the second series and reappeared after six months. Tumors on the other two cows progressed - but at a rate slower than that of tumors treated with neuraminidase.

310. BCG IMMUNOTHERAPY OF SUPERFICIAL BLADDER CANCER

Lamm, D. L., Thor, D. E., Smith, K. O., Harris, S. C., Radwin, H. M., Novicki, D. E., Winters, W. D., McMahan, C. A., University of Texas, Health Science Center at San Antonio, Medical School, Dept. of Surgery, *7703 Floyd Curl Dr., San Antonio, Texas*, 78284, United States of America

In an ongoing NCI contracted study, the authors have demonstrated that simultaneously administered intravesical and percutaneous BCG immunotherapy significantly (p equals 0.029, chi square) decreases the rate of recurrence of transitional cell carcinoma compared with controls. The duration of protection afforded by BCG immunotherapy, and the optimum dose, route, and frequency of BCG administration remain to be determined and are the primary objectives of this proposal. In addition, utilizing a murine bladder cancer model we have demonstrated that excessive BCG administration can impair the antitumor response or even enhance tumor growth, as has been seen by other investigators with different tumor models. It is critical, therefore, that the optimal dose and frequency of BCG immunotherapy be determined while cautiously avoiding excessive BCG administration. Since several biological systems are involved, it is likely that optimal BCG administration will vary from patient to patient. In an effort to develop appropriate monitors of the immune response which may be used to guide effective BCG immunotherapy, we propose to systematically monitor skin test reactivity to PPD, trychophytin, and candida antigens, leukocyte migration and adherence inhibition to bladder tumor and BCG antigens, humoral immunity to bladder tumor and BCG antigens as measured by solid phase radioimmunoassay, serum blocking factors, and serum anti-IgG factors.

311. IMMUNOTHERAPY OF BLADDER TUMORS IN A RAT MODEL SYSTEM

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The objective of this program is to establish and define a clinically relevant rat bladder tumor model for use in evaluating various immunotherapeutic and immunochemotherapeutic approaches to the treatment of carcinoma of the bladder.

Bladder tumors will be induced in Fischer 344 rats by oral administration of the chemical carcinogen, FANFT (N-(4-(5-nitro-2-furyl)-2-thiazolyl) formamide). The effects of such parameters as exposure interval and dietary lipid content will be investigated.

Bacillus Calmette-Guerin cell walls (BCG-CW) will be injected directly into the tumors to nonspecifically boost the host's immune respone to its tumor. The effect of the incorporation of mitomycin C, cis-platins or adriamycin into this protocol will also be investigated. Parameters of cellular and humoral immunity will be followed. These studies should be directly applicable to treatment of bladder cancer in humans.

Studies to date have established that 22 weeks of 0.2% FANFT will induce tumors in 100% of rats. Initial studies of the effects of intralesional injection of BCG-CW are currently being completed. However, it is clear that high dose BCG-CW combined with intravesical live BCG results in enhancement of tumor growth. An agarose microdroplet direct macrophage migration inhibition assay was developed as an in vitro assay of

cellular immunity. An in vivo delayed cutaneous hypersensitivity assay using footpad and ear testing sites was established. A solid phage RIA for detecting circulating antibody to BCG antigens was developed. The excellent correlation between these cellular and humoral assays suggests that immunologic responsiveness in subjects receiving nonspecific immunostimulation with BCG or its fractions may be effectively monitored by simple periodic serologic assay for circulating antibody to BCG antigens.

E. OTHER EXPERIMENTAL IMMUNOTHERAPY STUDIES AND BROAD PROGRAMS

312. MODULATION OF NATURAL IMMUNE REACTIVITIES AGAINST TUMORS

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The aim of the present project is to study: 1) The possible involvement of NK cells in in vivo antitumor resistance. 2) The mechanisms involved in the regulation of NK activity. These approaches will be relevant to better understanding of the biological role of such reactivity and the possible relationships with other immune mechanisms.

In vivo role of NK activity: We have recently performed in vivo experiments measuring the rapid cytolytic (0-4 hrs) activity of non-immune mice against 125IUdR-labelled tumor cells. Such reactivity correlates with the in vitro NK activity, as measured in various organs (spleen, lungs, etc.) by different criteria: a) Strain distribution; b) age dependency; c) thymus independency; d) susceptibility to the treatment with various agents: antineoplastic drugs (adriamycin, cyclophosphamide, DTIC, ARA-C), interferon and interferon-inducers (Poly I:C, C. parvum, Pyran copolymer, MSV, tumor cells), antimacrophage agents (Silica and Carrageenans), 17beta-estradiol, Dexamethasone, lethal doses of irradiation. The correlations indicate that NK cells could play a role in in vivo antitumor immunity.

MODULATION OF NK ACTIVITY: Various agents (Pyran copolymer, Poly I:C, C. parvum, adriamycin, etc.) have been found to modulate NK activity. Stimulatory and inhibitory effects can be observed varying dose and time of administration. Some agents (adriamycin, Pyran copolymer, C. parvum) induce marked depression of NK activity at the time when high activation of macrophages is observed. At this time recovery of NK activity can be obtained by removal of nylon-adherent cells from the spleen of depressed mice. The suppressive activity can be transferred in vitro by a cell population showing the following characteristics: a) Plastic-adherence; b) radioresistance (2000-5000R); c) ability to phagocytize latex particles; d) adherence or phagocytosis of carbonyl iron; e) resistance to treatment with anti-Thy I antiserum plus C. These results indicate that activated macrophages could be inhibitory for the lytic activity of NK cells.

313. ROLE OF CIRCULATING TUMOR ANTIGENS IN IMMUNOTHERAPY

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OBJECTIVES: Isolation of TSA, VRA, and AgB. Measurement and metabolism of these three categories of antigens in the circulation in hosts with regressing and progressing tumors. Analysis of the immune response to each category of antigen, but especially to TSA; the analysis includes a dissection of the cellular response (macrophage activity, T and B lymphocyte participation) and of the humoral response (a quantitative comprehension of antibody response according to Ig class). The use of tumor antigens to accelerate and augment destruction of tumor and survival of host. Role of different antigen components in tumor progression (blocking, tolerance, etc.). Effect of antigen on specific and nonspecific responses of host.

314. MECHANISMS OF CARCINOGENESIS

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The general objective is to increase our understanding of the biology of carcinogenesis.

Carcinogenesis is interpreted to include the whole series of events and processes that result in a fully developed, metastasizing neoplasm. A major portion of the work is therefore concerned with the interactions of a neoplastically transformed cell or clone with the host. In particular, emphasis will be given to the investigation of a newly discovered property of the immune response, namely specific tumor immunological stimulation. For this purpose both in vivo transplantation studies and in vitro cytotoxicity tests will be used. The 'nude' mouse in the germ-free state will be a principal tool. Additionally, the mechanisms of the immunological stimulation will be investigated and their role in the natural biology of cancer determined.

It is hoped that these studies will give insight into ways in which immunological stimulation can be prevented and immunological surveillance made more effective.

315. GENETIC ANALYSIS OF IMMUNOTHERAPY

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This is part of a broader project. A summary of this subproject is not available.

316. IMMUNOTHERAPY STUDIES OF SPONTANEOUS MALIGNANCY

Weiden, P. L., Storb, R., Thomas, E. D., Slichter, S. J., Tsoi, M., Sale, G., Deeg, H. J., Bensinger, W., Torok Storb, B., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington,* 98104, United States of America

We plan to continue to develop and assess the efficacy of novel approaches for the treatment of malignant disease based on studies in dogs with spontaneously occurring malignancies.

Studies in dogs with malignant lymphoma have shown that some dogs treated with chemotherapy, total body irradiation (TBI) and autologous marrow infusion can be cured of their disease but that most will ultimately develop recurrent lymphoma. We propose to determine whether recurrent lymphoma results from infusion of malignant cells in the autologous marrow or from failure of TBI to eradicate all malignant cells. We will investigate the mechanism of cure in dogs free of disease after therapy by determining whether such dogs are immune to autologous lymphoma cells. We also plan to study the graft-versus-tumor effect of allogeneic marrow transplantation using a new immunosuppressive agent, cyclosporin A.

In dogs with osteogenic sarcoma, we wish to test the hypothesis that growth of metastatic tumor can be inhibited by interference with hemostatic mechanisms. We also plan to assess whether the results of in vitro assays of cell-mediated and humoral immunity performed serially before and after amputation in dogs not given adjuvant chemotherapy correlate with in vivo events. We also will compare the effectiveness of conventional versus high dose methotrexate as cytoreductive chemotherapy administered before amputation.

We also plan to study the feasibility and effectiveness of positron emission tomography in tumor imaging.

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317. IMMUNOPREVENTION OF SPONTANEOUSLY OCCURRING NEOPLASMS

Dinowitz, M., Microbiological Associates Inc., *5221 River Rd., Bethesda, Maryland,* 20016, United States of America

OBJECTIVES AND APPROACH: Long-term holding of available strains of inbred mice and rats to define and characterize the incidence of spontaneous neoplasms and its relationship to the host's natural type C virus expression and natural immune defenses. Determine the natural incidence of type C RNA virus expression in neoplastic and normal tissues as determined by serological detection of antigens and in vivo and in vitro isolation of infectious virus from tissues of rodents with and without spontaneous neoplasia, and characterization of isolates by in

vivo and in vitro assays for biological activity and properties. Production of vaccines used for immunotherapy and immunoprevention of spontaneously occurring tumors. Vaccines to include viral, cellular, and subcellular preparations. Monitor and define the quality of the vaccine preparations. Define optimal conditions for production, handling, and storage of vaccines and define optimal route, dose, and schedule of immunization.

318. PRECLINICAL ANIMAL BONE MARROW TRANSPLANTATION

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OBJECTIVE: Provide technical and support services to perform granulocyte transplantation and immunotherapy studies for which protocols will be provided by the NCI. The veterinary and scientific investigators will meet on a weekly basis with the Experimental Hematology Section staff and discuss the formulation of experiments in maintenance and analysis of data arriving from outgoing protocols. Daily deliveries to and from the NIH, Building 10, and communication with the Experimental Hematology Section are required.

319. IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY OF ANIMAL TUMORS

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OBJECTIVE: Develop means of treating and controlling neoplasms by immunological methods.

APPROACH: Animals bearing naturally occurring or experimental neoplasms will be immunized with autogenous tumor cell vaccines. The tumors will be removed surgically and the cells used for the preparation of the vaccines. Cells will be treated by physical, chemical or by biological means to expose or release tumor specific antigens. The vaccine will be inoculated back into the subject and evaluation for enhanced immune response to the tumor cell antigens will be made. It is hoped that the stimulated immunity can destroy the residual tumor and protect against its reoccurrence.

PROGRESS: The immunological response to neoplastic growth requires the body to recognize and react against newly formed antigens on surfaces of tumor cells. Research in the past year included efforts to develop methods for identifying and eventually characterizing such antigens. Established tissue culture lines were used for this work. Cells were infected with viruses which replicate by budding from cell membranes. The viruses then were used as membrane markers. Rabbit antibodies specific for the virus were first incubated with the infected cells. This was followed by the addition of micron-sized plastic beads containing anti-rabbit antibodies which reacted with the antigen-antibody complexes bound to the cells. Thus the microbeads adhered to the cells identifying the sites of the marker antigens. This procedure has a number of advantages over the currently used fluorescent antibody approaches. It will facilitate studies in defining the nature of the cell antigens involved in tumor immunology. The work on therapeutic immunization for equine sarcoid has been continued. Autogenous vaccines were prepared for three naturally occurring cases. Successful results are indicated as the tumors have not recurred.

320. ANIMAL MODEL FOR SURGERY, CHEMOTHERAPY AND IMMUNOTHERAPY

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OBJECTIVES: This project is directed at the development of an animal tumor model which will elucidate principles in the timing and interaction of surgery, chemotherapy and immunotherapy.

PLAN: After establishment of a tumor line with a high metastatic potential, protocols which vary the time and dose of chemotherapy and immunotherapy with respect to surgery will be investigated. When maximally effective treatment regimens are established, the time course of corresponding immunologic events will be assayed in vitro.

METHODOLOGY: The animal model consists of a transplantable tumor in inbred mice which can be implanted in a site accessible for local 'cure' but with potential for systemic metastasis. Initial investigations with a methylcholanthrene induced sarcoma showed that the rate of metastasis was too low and the time course of the tumor too slow for practical, inexpensive laboratory work. The B-16 melanoma of C57/6/ mice has been a more useful tumor. A tumor cell suspension is passaged by injection into the thigh of the inbred mice. The limb is amputated and the animal is treated with various doses of cytoxan at varying times. Immunotherapy has not yet been added to the model.

FINDINGS: (1) Amputation of the limb prior to the appearance of palpable tumor will result in a significant number of cures. (2) Chemotherapy has a measurable effect on the primary tumor. (3) The effect of chemotherapy is dose related. (4) The combination of chemotherapy and amputation at the time the tumor becomes palpable is better than either modality alone. (5) Early chemotherapy is more important than amputation of the primary but both are necessary for cure. (6) If performed too late, complete excision has no survival advantage over incomplete excision.

These findings suggest that if metastases are likely but not demonstrable, then initial, aggressive chemotherapy should take precedence over excision of the primary.

321. A NEW MODEL FOR CHEMO-IMMUNOTHERAPY OF LYMPHOMA

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Two unique competencies and findings from this laboratory will be used to study therapy of murine lymphomas: 1) Evidence that a purified, non-oncogenic virus, which is intimately associated with development of lymphomas, is effective in prophylaxis of transplanted lymphoma, 2) Evidence that a chemotherapeutic regimen utilizing cyclophosphamide and N-acetylcysteine can bring about a remission of growth of the same transplanted lymphoma. We plan to determine whether we can produce therapy, as compared to prophylaxis, of the lymphoma using virus alone or as an adjunct to chemotherapy. Preliminary evidence indicates that virus therapy is successful with low tumor loads of transplanted cells. In order to make these findings generalizable to other system, use of virus therapy with 1) higher tumor loads, 2) tumors from other sources, and 3) recurring tumors after chemotherapy will be studied. Use of a single high dose of a chemotherapeutic agent alone or in combination with an immunotherapeutic agent, as compared to repeated smaller doses that are used more conventionally, is both cost and care efficient if the side effects of the higher dose can be minimized as is proposed here.

322. INDUCTION OF INCREASED IMMUNOLOGICAL REACTIVITY IN MICE

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This is part of a broader project. A summary of this subproject is not available.

323. MODELS FOR TUMOR IMMUNOTHERAPY AND IMMUNOPREVENTION (MICE, RATS)

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This is part of a broader project. A summary of this subproject is not available.

324. RADIOTHERAPY AND IMMUNOTHERAPY (MICE)

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This is part of a broader project. A summary of this subproject is not available.

INVESTIGATOR INDEX

Asronson, S. 251
Aisner, J. 11
Albano, W.A. 301
Albino, A.P. 2
Alley, R.D. 123
Almaraz, R. 42, 295
Almeida, A.B. 88
Amery, W.K. 54
Amgwerd, R. 76
Amick, C. 150
Andersen, P. 68
Anderson, B. 20
Anderson, L. 210
Antila, P. 57
Arai, K. 237
Ariyan, S. 115
Ashley, M.P. 174, 187
Aust, J.B. 104
Bach, M.I. 261
Baker, A.R. 187
Baldwin, R.W. 89
Baley, R. 273
Ballou, B.T. 92
Bannayan, G.A. 104
Banowsky, L.H. 82
Baral, E. 144
Barlozzari, T. 312
Barr, C. 147
Barrett, A.J. 19
Barth, R.F. 218
Bartocci, A. 221, 312
Bast, R.C. 244
Baue, A.E. 58
Bejamini, E. 236
Bennett, J.A. 51, 123
Bensinger, W. 316
Beran, M. 20
Bernstein, I.D. 245
Beschorner, W.E. 264
Beska, F. 4
Bhayana, J. 63
Bier, J. 187
Bier, M. 203
Black, M.M. 71
Biaese, R.M. 148
Blair, A.H. 248
Blatt, J. 148
Blievernicht, S. 118
Bloksma, N. 216
Blomgren, H. 144
Blonk Van der Wijst, J. 54
Blumenstock, F.A. 65, 223
Bodey, G.P. 152
Boedeker, B. 220
Bolhuis, R.L. 54
Bollum, F. 148
Bolognesi, D.P. 72, 254, 315
Bolognesi, L.T. 88
Borden, E.C. 162
Bortin, M.M. 258, 259
Bosio, N. 68
Bottomley, R.H. 9, 28, 29, 93
Bowles, C. 251
Bowles, C.A. 318
Brailovsky, C.A. 217
Brandt, A.E. 238
Braun, D.P. 64
Brehmer, W. 202
Bridges, C.J. 203
Bridgman, A.H. 128
Broder, S. 148
Brody, A.W. 301
Browder, W. 214
Brown, C.A. 175
Brown, J. 42, 295
Brummer, E. 279
Bunn, P.A. 253
Burger, D.R. 143
Burgess, M.A. 152
Burnstein, T. 319
Butti, G. 134
Byrom, N. 6
Bystryn, J. 13
Callahan, T. 148
Cantrell, J.L. 191, 200, 202, 205
Carotti, C. 312
Carter, D. 58
Castello, M.A. 132
Catravas, G.N. 276
Cesarini, C. 312
Chang, J. 231
Chaperon, E.A. 301
Chau, J.C. 122
Chee, D.O. 107
Cheever, M.A. 260
Chirigos, M.A. 221, 226
Choe, B. 136
Choi, O.S. 150
Chretien, P. 130
Clarke, A.G. 89
Clerico, A. 132
Cockerell, G.L. 180, 181
Cohen, E.P. 231
Cohen, M.H. 253
Collins, J. 291
Collins, J.J. 72, 255
Colnaghi, M.I. 242
Coltman, C.A. 12, 30, 41, 73, 129, 141
Colton, C. 203
Cormack, D.V. 250
Corsini, A.C. 125
Costanzi, J.J. 156
Costea, N. 289
Cox, J.D. 56
Crispen, R.G. 145
Crum, E.D. 177
Cruz, A.B. 104
Cummins, C.S. 188
Curiel, R. 139
Curtis, A. 58
D'Auria, A.E. 8
de Carli, H.O. 70, 99
De Kernion, J.B. 178, 190
de los Santos, J. 8
de Weger, R.A. 270
Deeg, H.J. 316
Defendi, V. 281
deKoning, J. 26
Delamore, I. 18
DeLanney, L.E. 314
DellaPorta, G. 242
den Otter, W. 270
Dent, P.B. 1, 14
Di Luzio, N.R. 214
Diez, E. 68
Dinowitz, M. 211, 317
Distasio, J.A. 305
Doe, W.F. 98
Donlon, J. 148
Dooren, L.J. 26
Dorlhiac, P. 38
Dorsman, B. 98
Dray, S. 273, 286
Dwivedi, C. 222
Eberlein, T. 42, 295
Eells, R. 113, 126
Eilber, F.R. 149
Ekert, H. 22, 31, 131
Emeson, E.E. 257
England, R.B. 309
EppingerHelft, M. 32
Ervin, T. 91
Essex, M.E. 293
Ewel, C. 220
Facciolo, F. 49
Farr, R.S. 25
Fefer, A. 260, 267
Feldman, J.D. 313
Finkelstein, M. 283
Fischer, J.J. 58
Fischer, S. 229
Fish, D. 251
Fisher, B. 79, 110, 196
Fisher, R.I. 7, 253
Fishman, M. 195
Forbes, J.T. 275
Forrest, D. 138
Foster, E.D. 123
Foster, L. 278
Franco, P. 292
Franklin, E.C. 277
Frappier Davignon, L. 34
Freeman, C.B. 18
Frei, E. 91
Froese, G. 250
Frost, P. 269
Gage, A. 63
Gandbhir, L. 150
Gangal, S.G. 120
Gardner, M.B. 251
Gergely, P. 142
Ghose, T.T. 248
Gilbertson, S.R. 71
Gilden, R. 251
Gilliland, C.D. 215
Gillis, C.R. 124
Girija, P. 87
Gitsch, E. 85
Gizycka, D. 47
Glickman, M. 115
Goldberg, E.H. 271
Goldberg, E.P. 205
Goldenberg, G. 122
Goldman, L.I. 103
Golinger, R.C. 103
Gorczynski, R.M. 246
Gorecka Tisera, A. 173, 189
Goren, M.B. 199

Gosti, A. 312
Granatek, C.H. 202, 205
Grandval, C.M. 97
Gray, G. 147
Gray, G.R. 169
Green, S. 198
Greenberg, M. 56
Greenberg, P.D. 260
Grifoni, V. 39
Grohn, P.T. 74
Grozea, P.N. 9, 28, 29, 93
Gunduz, N. 196
Gunn, W. 56
Gutterman, J.U. 164
Haije, W. 54
Hakala, T. 57
Hakala, T.R. 92
Hakama, M. 57
Hammerling, U.G. 290
Hande, K.R. 10
Hanjani, P. 229
Hardy, W.D. 212
Hardy, W.R. 140
Hargis, B. 222
Harris, J.E. 64
Harris, R. 18
Harris, S.C. 303, 310, 311
Hartley, J. 174
Haskell, C.M. 50
Hast, R. 20
Hawrylko, E. 170
Hayakawa, K. 48
Hayata, Y. 48, 299
Heck, F.C. 309
Heinonen, E.O. 74
Hellstrom, K.E. 323
Hemstreet, G.P. 96
Henderson, E.S. 37
Henney, C.S. 176
Herberman, R.B. 266
Hersh, E.M. 151
Heston, W.D. 247
Hiles, R. 6
Hirshaut, Y. 154, 161
Ho, J.H. 122
Hobbs, J. 132
Hobbs, J.R. 6, 138
Hodes, R.J. 7
Hofhuis, F.M. 216
Hojo, K. 109
Holcenberg, J.S. 218
Holland, J.F. 21
Holmes, E.C. 50, 67
Hop, W.C. 54
Houghton, A.N. 2
Hovis, J. 251
Hubay, C. 80
Huberman, M. 150
Huebner, R.J. 251
Humphrey, G.B. 157, 285
Hunter, J.T. 187, 262
Hunter, R.L. 228
Hurvitz, A.I. 71
Hussain, M. 123
Hussein, K.K. 9, 28, 29, 93
Hyatt, C. 42, 295
Iglehart, D.J. 298
Ihde, D.C. 253
Israili, Z.H. 172
Jacobs, D.M. 206
Jamra, M. 38
Jeljaszewicz, J. 208
Jewell, W.R. 163
Jones, G. 205
Jose, D.G. 31
Joseph, R. 229
Joyce, J.R. 185, 241
Juillard, G.J. 94
Jungi, W.F. 76
Kahan, B.D. 234
Kaiser, C.W. 150
Kalmar, L. 142
Kamphuis, R.P. 26
Kaplan, A.M. 118
Kapp, D.S. 58
Katz, L. 35
Kausel, H.W. 123
Kay, H.E. 19
Kelleher, P.J. 25
Kelloff, G.J. 211
Kende, M. 211
Kenyon, S.J. 297
Kessler, I.I. 146
Khomasurya, B. 246
Kitahama, A. 214
Klefstrom, P.S. 74
Kleinschuster, S.J. 186, 187, 308
Knapp, R.C. 95, 244
Knapp, W. 85
Knerich, R. 134
Kochie, P. 130
Kong, A.S. 207
Kongshavn, P.A. 263
Koops, H.S. 5
Koyama, Y. 109
Krishnamurthi, S. 87
Krohn, K. 57
Krzysko, R. 47, 55
Kulczycki, A. 224
Kun, L. 56
Kuribayashi, K. 176
Kwan, H.C. 122
Kysela, T. 4
La Greca, G. 132
Labadie, E.L. 287
Ladisch, S.K. 148
Lamm, D.L. 81, 82, 137, 310, 311
Lampkin, B.C. 36
Lang, I. 142
Langlois, A.J. 72
Lantz, B. 20
Lapp, W.S. 263
Laursen, M.L. 230
Lavergne, B. 34
Lawrence, H.S. 282
Lawrence, W. 118
Leahy, M.A. 23
Leventhal, B.G. 33
Levy, L. 321
Liao, S.K. 1, 14
Lisv, A. 199
Lilliehook, B. 144
Lininger, L. 123
Livingston, P.O. 2
Locatelli, D. 134
Lochman, I. 4
Loke, J. 58
Loose, L.D. 168, 197
Lopez, D.M. 305
Lotze, M. 42, 295
Lozzio, B.B. 266
Lozzio, C.B. 266
Lukaszewicz, M. 47, 55
Lynch, H. 251
MacEwen, E.G. 71
Machado, E.A. 266
Machalek, J. 4
MacRae, S. 246
Mahaley, M.S. 135
Mak, A.C. 54
Maki, T. 274
Mancuso, G. 132
Mannick, J.A. 91, 288
Marelli, O. 292
Margraf, H.W. 224
Mate, M. 8
Matthay, R.A. 53, 58
Matthews, J. 31
Matthews, R.N. 31
Mayer Scogna, D. 229
McCulloch, P.B. 14
McIlduff, J.B. 123
McKelvey, E.M. 152
McKneally, M. 65, 223
McKneally, M.F. 123
McLaren, J. 113, 126
McLaughlin, C.A. 191, 200, 202, 205
McMahan, C.A. 310
McNamee, R. 214
McSwan, N. 124
Megirian, R. 65, 168, 197, 223
Melino, G. 132, 138
Menard, S. 242
Mendeloff, G.L. 52
Mestre, M. 75, 108, 114, 125
Metchell, J.M. 276
Milgrom, F. 235
Milner, E.C. 200
Minden, P. 25, 202
Mineko, N. 75
Mineo, T.C. 49
Minna, J.D. 253
Mira, J.G. 137
Mirra, A.P. 60
Mitchell, M.S. 27, 115
Miziara, J.E. 60
Modesto, N. 108
Mokyr, M. 273
Morantz, R.A. 265, 307
Moriya, Y. 109
Morrison, M. 195
Morrison, M.K. 136
Morse, S.I. 207
Morton, D.L. 69, 153
Murahata, R.I. 191
Murasko, D.M. 209
Murray, J.L. 9, 28, 29, 93
Nagarajan, B. 87
Nair, P.N. 120
Nakamura, M.J. 200
Neidhart, J.A. 90
Neifeld, J.P. 118
Neilan, B. 229
Nekam, K. 142
Neshat, A.A. 111, 128
Nicolin, A. 211
Nicolin, A.N. 292
Niell, H.B. 61
Nigam, V.N. 217
Nitta, K. 213
North, R.J. 192
Novicki, D.E. 310
Oettgen, H.F. 2, 83, 159, 322
Ogawa, I. 48, 299
Oho, K. 299
Old, L.J. 2
Older, T.M. 123
Oldham, R.K. 10, 62, 275
Oliveira Filho, J.A. 75, 108, 114, 125
Olkowski, Z. 113, 126
Olsen, R.G. 294
Ommaya, A.K. 130
Order, S.E. 155, 249
Osteen, R.T. 320
Outzen, H.C. 232
Owen, L.N. 296, 302
Pack, F.D. 185, 241
Paoletti, P. 134
Papac, R.J. 58, 115
Papademitriou, V. 221
Papermaster, B.W. 215
Paque, R.E. 311
Parker, C.W. 224
Parker, R. 202

Parker, R.A. 200
Patel, V.C. 130
Pavlidis, N. 221
Pavlovsky, S. 32
Peavy, D.L. 179
Periti, P. 105
Peroni, D. 292
Peruzzi, L. 312
Petersen, R. 229
Petranyi, G. 142
Philpott, G.W. 224
Piccardo, C. 39
Pichler, W. 148
Pierce, K.R. 185, 241
Pierri, I. 39
Pincus, J.H. 238
Pinsky, C.M. 84, 160
Pitelka, F.A. 109
Pitt, A. 203
Plate, J.M. 64
Pontes, J.E. 136
Poplack, D. 130
Poplack, D.G. 148
Portelance, V. 166
Powles, R.P. 19
Pozzi, D. 38
Preheim, L.C. 45
Prehn, L.M. 314
Prehn, R.T. 314
Priario, J.C. 8
Proctor, J.W. 173, 189
Radwin, H.M. 82, 310
Rainer, H. 85
Raisanen, L. 57
Raker, J. 121
Ramming, K. 50
Ramming, K.P. 86, 101, 102
Rangala, N. 56
Rao, R.S. 120
Rapp, H.J. 174, 187, 201, 262
Ratajczak, H. 196
Raymond, L. 58
Rea, S. 49
Reaman, G.H. 148
Reed, J. 130
Reed, R.C. 104
Reid, C. 6
Reid, G.M. 150
Reif, A.E. 256
Reinisch, C.L. 227
Reizenstein, P. 20
Rengachary, S.S. 265
Restbergs, V. 70, 99
Retsas, S. 138
Reynolds, H.Y. 58
Rhim, J. 251
Ribi, E.E. 191, 200, 202, 205
Riccardi, C. 312
Ricci, C. 49
Rice, J. 187
Rice, M.S. 23
Richie, J.P. 91
Richman, S. 202
Ritz, J. 244
Robbins, R. 229
Roberts, D. 206
Robinson, E. 106
Robustelli della Cuna, G. 134
Roloson, G.J. 72
Rose, N.R. 136
Rosenberg, S.A. 7, 42, 187, 295
Rosenspire, A. 206
Rosenstein, M. 42, 295
Rosenthal, K.L. 233
Roszkowski, W. 208
Rothbarth, P.H. 26
Rudbach, J.A. 200
Saba, T.M. 65, 223
Sacco, O. 49
Sadowsky, D. 130
Saiers, J.H. 140
Saiki, J. 140
Sale, G. 316
Samman, P.D. 138
Sanani, S. 40, 43, 77
Sangiuolo, A. 39
Santoni, A. 312
Sarra, F. 292
Sartiano, G.P. 59
Sasaki, J. 191, 200
Sasaki, T. 213
Scanlon, E.F. 78
Scarsi, P.G. 39
Schmidt, D.D. 284
Schneider, C.A. 60
Schwartzman, S.M. 191, 202, 205
Schwarz, S. 42, 295
Scibienski, R.J. 204, 236
Scornik, J.C. 252
Scudeletti, M. 39
Seeger, R.C. 133
Seigler, H.F. 16
Senn, H. 76
Serrou, B. 167
Shain, S.A. 82
Shanta, V. 87
Sharma, B.S. 24
Shifrine, M. 183
Shu, S. 262
Siessmann, K. 273
Silveira, G.G. 88
Silveira, J.G. 88
Silverman, M.A. 46
Singal, D.P. 1
Slichter, S.J. 316
Sljivic, V.S. 175
Sloan, B. 237
SlowikGabryelska, A. 47, 55
Smalley, R.V. 229
Smith, K.O. 310
Smyth, J. 148
Solomon, B. 203
Song, C.W. 324
Spaulding, M.B. 40, 43, 77
Spiess, P. 42, 295
Spilg, W.G. 124
Springer, G.F. 191
Stabile, B.E. 102
Stack, B.H. 124
Stansel, H.C. 115
Steele, G. 91
Steele, G.D. 288
Stoer, H.W. 194
Steerenberg, P. 262
Stefani, S.S. 127
Steinitz, R. 35
Stephenson, J.R. 211
Stolbach, L.L. 150
Storb, R. 316
Strain, S.M. 200
Strander, H. 44
Strausser, J. 42, 295
Strong, E.W. 117
Stylos, W. 221
Stylos, W.A. 226
Suen, J.Y. 112
Sugimoto, T. 174
Suit, H.D. 121
Sukumar, S. 174, 187
Suurkula, M. 268
Swartzwelder, F. 206
Symonds, E.M. 89
Szmigielski, S. 208
Taira, O. 48, 299
Takayama, K.K. 210
Takita, H. 63, 66
Tarkkanen, J.O. 74
Tatra, G. 85
Tauro, G.P. 31
Temple, C. 98
Templis, L.L. 258
Terman, D.S. 203
Terry, W.D. 7
Testorelli, C. 292
The, T.H. 5
Theilen, G. 240
Theilen, G.H. 184
Thomas, E.D. 165, 316
Thor, D.E. 219, 310
Thorbecke, G.J. 280
Tognella, S. 39
Tokuda, S. 271
Tom, B.H. 100
Tompkins, W.A. 239
Toogood, I.R. 23
Torok Storb, B. 316
Toubiana, R. 205
Tracey, D.E. 225
TreurnietDonker, A.D. 54
Trigg, M. 148
Trippe, N. 60
Truitt, R.L. 258, 259
Tsoi, M. 316
Tutschka, P.J. 264
Tuttle, R.T. 191
Uden, A. 20
Uotila, A. 57
Uren, J.R. 222
Vaage, J. 304, 306
Valentine, F.T. 15
van der Giessen, M. 5
Van Echo, D.A. 11
van Loveren, H. 270
Van Scott, E.J. 158
Vandenbark, A.A. 143
Vasey, D. 89
Vaughan, K. 248
Veit, B.C. 195
Veronese, F. 211
Veronesi, F. 292
Veronesi, U. 3
Vetto, R.M. 143
Vider, M. 229
Von Eschen, K.B. 200, 202, 205
Vosika, G. 202
Vosika, G.J. 147
Vossen, J.M. 26
Vredevoe, D.L. 321
Wagner, G. 85
Wainberg, M.A. 182
Wallace, H.W. 237
Walters, C. 130
Wang, B.S. 91, 288
Wang, C.C. 121
Wara, W.M. 116
Waters, K.D. 22, 31, 131
Weber, P.B. 65, 223
Weiden, P.L. 316
Weiss, J. 221
Weiss, L. 306
Wells, S. 200
Wepsic, H.T. 171
Westbrook, K.C. 112
Wetmore, S.J. 112
Wexler, H. 42, 295
Wheat, R. 191
White, R. 296, 302
White, R.G. 124
Wiernik, P.H. 11
Willers, J.M. 216
Williams, J.C. 200
Willis, I. 172
Wilson, J.F. 56
Wilson, R. 320

Wilson, R.E. 91
Winkelhake, J.L. 218
Winters, W.D. 310, 311
Wise, H. 90
Witz, I. 211
Wolmark, N. 196
Wood, G.W. 307
Wright, P.W. 17
Yamamura, Y. 243
Yarkoni, E. 187, 201
Yonemoto, R.H. 107
Yron, I. 42, 295
Zachrau, R.E. 71
Zarling, J.M. 261, 272
Zbar, B. 174, 187, 202
Zellen, M. 91
Zigbelboim, J. 119, 191, 193
Zinkernagel, R.M. 233
Zahrie, S.R. 18
Zwilling, B.S. 300

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